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CONTENTS OF VOLUME I

JANUARY, 1925. NUMBER 1

INTRANUCLEAR INCLUSIONS IN EXPERIMENTAL HERPETIC LESIONS OF RABBITS. *Ernest W. Goodpasture.* Plate I 1

THE AXIS-CYLINDERS OF PERIPHERAL NERVES AS PORTALS OF ENTRY TO THE CENTRAL NERVOUS SYSTEM FOR THE VIRUS OF HERPES SIMPLEX IN EXPERIMENTALLY INFECTED RABBITS. *Ernest W. Goodpasture.* Plates II–IV 11

THE PATHWAYS OF INFECTION OF THE CENTRAL NERVOUS SYSTEM IN HERPETIC ENCEPHALITIS OF RABBITS CONTRACTED BY CONTACT; WITH A COMPARATIVE COMMENT ON MEDULLARY LESIONS IN A CASE OF HUMAN POLIOMYELITIS. *Ernest W. Goodpasture* 29

CERTAIN FACTORS DETERMINING THE INCIDENCE AND SEVERITY OF HERPETIC ENCEPHALITIS IN RABBITS. *Ernest W. Goodpasture* 47

RIEDEL'S STRUMA ASSOCIATED WITH REMNANTS OF THE POST-BRANCHIAL BODY. *Louise H. Meeker.* Plates V–X 57

HETEROTOPIA OF THE BONE MARROW WITHOUT APPARENT CAUSE. *E. R. Saleeby.* Plates XI, XII 69

MICROGLIA AND THE PROCESS OF PHAGOCYTOSIS IN GLIOMAS. *Wilder Penfield.* Plates XIII–XX 77

THE FORMATION OF MACROPHAGES, EPITHELIOID CELLS AND GIANT CELLS FROM LEUCOCYTES IN INCUBATED BLOOD. *Margaret Reed Lewis.* Plate XXI 91

STUDIES ON BLOOD FIBRIN. ITS QUANTITATIVE DETERMINATION; NORMAL FIBRIN VALUES, AND FACTORS WHICH INFLUENCE THE QUANTITY OF BLOOD FIBRIN. *E. W. Schultz, J. K. Nicholes and J. H. Schaefer* 101

THE RELATION OF CHRONIC POISONING WITH COPPER TO HEMOCHROMATOSIS. *F. B. Mallory* 117

MARCH, 1925. NUMBER 2

EVIDENCE OF REGENERATION OF PANCREAS IN AN INSULIN TREATED CASE OF DIABETES. *Gladys L. Boyd and W. L. Robinson.* Plate XXII 135

THE HISTOPATHOLOGY OF KALA-AZAR IN THE HAMSTER, MONKEY, AND MAN. *Henry Edmund Meleney.* Plates XXIII–XXVIII 147

PHAGOCYTOSIS OF ERYTHROCYTES IN THE BONE MARROW, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA. *Francis W. Peabody and G. O. Broun.* Plates XXIX, XXX 169

Concerning the Microscopic Structure of the Hypophysis Cerebri in Acromegaly (Based on a Study of Tissues Removed at Operation from 35 Patients). *Percival Bailey and Leo M. Davidoff.* Plates XXXI–XL 185

The Pathology of the Hypophysis. I. The Presence of Abnormal Cells in the Posterior Lobe. *J. P. Simonds and W. C. Brandes.* Plate XLI 209

The Hypertrophy of the Submaxillary Gland in the Albino Rat Following Parathyroidectomy. *Joseph L. Appleton* 217

Hematocele of the Wall of the Atrium Following Cardiac Puncture. *Edwin W. Schultz.* Plate XLII 225

The Hydatid Cyst. The Micro-Chemical Reactions of the Hydatid Cyst Wall. *Gordon Cameron and A. S. Fitzpatrick* 227

The Origin of Adenomatous Goitre. *B. S. Kline.* Plates XLIII, XLIV 235

The Etiology of Acute Appendicitis. *Shields Warren* 241

MAY, 1925. NUMBER 3

Experimental Glomerulonephritis. *E. T. Bell, B. J. Clawson and T. B. Hartzell.* Plates XLV–XLVIII 247

The Fibroma of the Acoustic Nerve. *Victor C. Jacobson.* Plates XLIX–LII 259

The Pathology of the Hypophysis. II. Lymphocytic Infiltration. *J. P. Simonds and W. W. Brandes.* Plate LIII 273

An Adenocarcinoma Resembling the Thyroid Gland. *Wm. H. Feldman.* Plate LIV 281

Tissue Changes after Experimental Deep Roentgen Irradiation. *Anatole Kolodny.* Plate LV 285

Studies on Mitochondria. I. The Changes Occurring During Experimental Thyroid Hyperplasia and its Involution with Iodine. *David P. Seecof* 295

The Origin of the Mononuclear Phagocytes of Peritoneal Exudates. *Frank A. McJunkin* 305

Studies on Filterable Viruses. I. Cultivation of Vaccine Virus. *Frederic Parker, Jr., and Robert N. Nye* 325

Studies on Filterable Viruses. II. Cultivation of Herpes Virus. *Frederic Parker, Jr., and Robert N. Nye* 337

JULY, 1925. NUMBER 4

Studies on Endothelial Reactions. IX. The Formation of Reticulum in the Lesions of Experimental Tuberculosis in Rabbits. *Nathan Chandler Foot.* Plates LVI, LVII 341

The State of the Cardiac Muscle in Hypertrophy and Atrophy. *Howard T. Karsner, Otto Saphir and T. Wingate Todd.* Plate LVIII 351

THE HISTOLOGICAL ALTERATIONS OF THE PANCREAS IN CHRONIC PASSIVE CONGESTION. *William C. VonGlahn and Robert Chobot.* Plates LIX, LX 373

CHOLESTEROSIS OF THE GALL BLADDER. *Stanley H. Mentzer* 383

ARACHNID INFECTION IN MONKEYS (Pneumonyssus Foxi of Weidman). *Ferdinand C. Helwig.* Plates LXI, LXII 389

MUCOCELE OF THE APPENDIX WITH GLOBOID BODY FORMATION. *Gibbs Milliken and C. A. Poindexter.* Plates LXIII, LXIV 397

THE EFFECT OF CERTAIN METABOLIC CHANGES UPON THE AORTA OF RABBITS AND GUINEA PIGS. *Otto Saphir* 403

STUDIES ON PNEUMONIA FOLLOWING NASO-PHARYNGEAL INJECTIONS OF OIL. *G. F. Laughlen.* Plate LXV. 407

THE PATHOLOGY OF DIABETES, WITH SPECIAL REFERENCE TO PANCREATIC REGENERATION. *Shields Warren and Howard F. Root.* Plate LXVI 415

THE OCCURRENCE OF RETICULUM IN TUMORS. *N. C. Foot and H. A. Day.* Plates LXVII–LXXI 431

SEPTEMBER, 1925. NUMBER 5

INTRANUCLEAR INCLUSIONS IN VISCERAL DISEASE. *William C. VonGlahn and Alwin M. Pappenheimer.* Plates LXXII, LXXIII . . . 445

CONGENITAL HEART DISEASE WITH PARTIAL SITUS INVERSUS, ABSENCE OF INFERIOR VENA CAVA, AND OTHER ANOMALIES. *A. James Miller.* Plates LXXIV–LXXVII 467

PROGRESSIVE ALCOHOLIC CIRRHOSIS. *Ernest M. Hall and W. Ophüls.* Plates LXXVIII–LXXXI 477

OBSERVATIONS ON FOCAL TUBERCULIN HYPERSENSITIVENESS IN AN INFECTED ORGAN PREVIOUS TO A GENERAL SENSITIZATION. *Fred W. Stewart.* Plates LXXXII–LXXXV 495

GROSS AND HISTOLOGIC CHANGES IN THE EDEMA OF PARAPHENYLENEDIAMINE. *M. L. Tainter and E. M. Hall.* Plate LXXXVI 503

MECHANICAL IRRITATION AS ETIOLOGIC FACTOR OF CANCER. Clinical Observation. *Nicholas M. Alter.* Plates LXXXVII, LXXXVIII . 511

NEUROBLASTOMA OF THE INTESTINE. *Saul Alfred Ritter.* Plates LXXXIX–XCI 519

SCIENTIFIC PROCEEDINGS OF THE TWENTY-FIFTH ANNUAL MEETING OF THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS 527

NOVEMBER, 1925. NUMBER 6

A STUDY OF RABIES, WITH REFERENCE TO A NEURAL TRANSMISSION OF THE VIRUS IN RABBITS, AND THE STRUCTURE AND SIGNIFICANCE OF NEGRI BODIES. *Ernest W. Goodpasture.* Plates XCII, XCIII . . 547

DIFFUSE TUMORS OF THE MENINGES. *William Boyd.* Plates XCIV, XCV 583

Splenomegaly (Type Gaucher) and Lipoid-Histiocytosis (Type Niemann). *William Bloom.* Plates XCVI–C 595

Ependymomas of the Lateral and Fourth Ventricles of the Brain. *Edwin F. Hirsch and Arthur R. Elliott.* Plates CI–CIII . 627

Researches on Vaccine Virus *in Vitro* with Special References to its Affinity for Nerve Tissue. *Norimasa Hirano* 635

Spontaneous Central Nervous System Lesions in the Laboratory Rabbit. *Lauretta Bender* 653

Experimental Production of Gliosis. I. Effects on the Nervous System of the Rabbit of Intravenous and Intraspinal Injections of Cholesterol Emulsion. *Lauretta Bender* 657

Experimental Production of Gliosis. II. Reaction of Brain Tissue to the Lipoid Fractions and to the Residue of Brain Extracts. *Lauretta Bender.* Plate CIV 667

A Multinucleated Liver Cell Carcinoma. *H. S. Rowen and F. B. Mallory.* Plates CV–CIX 677

A Correction. *N. C. Foot* 681

THE AMERICAN JOURNAL OF PATHOLOGY

Volume I | January, 1925 | Number 1

INTRANUCLEAR INCLUSIONS IN EXPERIMENTAL HERPETIC LESIONS OF RABBITS *

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Within cells of early lesions experimentally induced in rabbits with herpetic virus there occur characteristic intranuclear inclusions the presence of which, because of their constancy and their uniformity of structure and staining properties, serves to establish histologically the diagnosis of the herpetic nature of a given lesion in an animal inoculated with this virus. Similar intranuclear inclusions occur within epithelial cells of fresh vesicles of herpes simplex in the human.

In this respect the virus of herpes is similar to a large group of so-called filterable viruses which induce within cells of an infected tissue certain structures which are proper to the lesions characteristic of each. To this group belong the diseases smallpox, rabies, molluscum contagiosum, trachoma, Geflügelpocke, varicella and others. In most of these infections the corresponding "inclusions" are found within the cytoplasm of cells. The inclusions of herpes, however, occur only within nuclei. Lipschütz [1] and others have regarded the cellular inclusions of these various diseases as composed in part at least of the specific virus itself, and formed as a result of the proliferation of the infectious agent within the cell affected. While this hypothesis is a most useful one and has much to support it, further evidence is necessary to establish its truth. One cannot rely entirely upon the morphology of minute components of such structures to establish with certainty their parasitic nature. But whether or not

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it will eventually be proved that the inclusions and the virus are identical, nevertheless much can be learnt by clearly recognizing a constant association of a characteristic type of inclusion with any particular infection, and by identifying it with the lesion so that a diagnosis of the infection on this basis may be possible. In the study of such inclusions evidence for or against their hypothetical relation to the virus will from time to time accumulate.

Lipschütz,[2] Luger and Lauda,[3] and Lauda[4] have described so clearly the characteristics of the intranuclear inclusions of herpetic lesions, and our own investigations[5] with the virus of herpes simplex have so completely confirmed and extended their observations that it has been surprising to observe in certain recent publications a failure to recognize their uniform occurrence and an underestimation of the morphological importance of these bodies in herpetic lesions, aside from any consideration of their possible identity with the virus. Thus Cowdry and Nicholson[6] summarize the result of their study of various granulations in herpetic lesions: "It is our belief that the inclusions which are so abundant in herpetic lesions do not represent a concrete class of granulations *sui generis* but that they are of variable composition and are derived from several sources." They describe the nuclear inclusions of Lipschütz, but do not consider them any more specific for the herpetic lesion than various intracellular and extracellular granules obviously of degenerative origin. The issue has been obscured by the description of ill-defined, cytoplasmic and nuclear granules, by da Fano,[7] Levaditi[8] and others which either have nothing to do with the inclusions so clearly defined by Lipschütz or are so confused as to make impossible a judgment of their relation to them.

A clearer general recognition of the characteristics of herpetic intranuclear bodies is necessary to a more exact understanding of the nature of the virus, of the lesions it produces and of its behavior as an infectious agent.

Identification of these intranuclear structures as described by Lipschütz has been very valuable in investigations of local herpetic lesions[9] and of the manner in which the virus progresses from the periphery to infect the central nervous system, and it has seemed therefore advisable to restate the characteristics of these inclusions and to record our impressions as to their composition, structure and mode of formation.

The characteristic herpetic inclusion is confined strictly to the interior of the nucleus. We have found no type of granulation or other structure in the cytoplasm of cells or situated extracellularly which has any specific or constant relation to a herpetic infection.

The herpetic inclusions are to be distinguished from the preformed structures or products within the normal nucleus. They are distinct from the nucleoli and the products of nucleolar disintegration. Both morphologically and tinctorially it can be determined that the nucleoli play no morphological part in the formation of the herpetic inclusions. Nucleoli showing various stages of degeneration may be recognized in ganglion cells of the central nervous system in clear contrast to the herpetic bodies in hematoxylin-eosin preparations. The nucleoli may appear intensely red in preparations stained with carbol-fuchsin and counterstained with Loeffler's methylene blue and differentiated in alcohol. In such a preparation the herpetic bodies do not stain at all or may be faintly blue depending upon the degree of differentiation. The nucleoli stain intensely with acid fuchsin, in an acid fuchsin-methyl green preparation, while the herpetic bodies remain unstained or appear faintly green.

Nuclear chromatin is readily distinguishable from the material composing the inclusions by its avidity for basic dyes. In the disintegrating nucleus granules of chromatin still stain a deep blue after the eosin-methylene blue or Giemsa method.

The herpetic intranuclear inclusions are of a different structure and composition from the two nuclear constituents, chromatin and nucleolus. They are not to be distinguished in earliest stages, however, from material precipitated from the nucleoplasm by fixation in Helly's fluid. This is to be seen especially well in large motor ganglion cells of the central nervous system in which nucleoli are prominent and sharply circumscribed and nuclear chromatin is not abundant. In the interior of the nuclei of these cells fixed immediately after death by direct injection of Helly's fluid into the carotid arteries there are one or more groups of amorphous finely granular material, which seem to have precipitated about certain centers (Fig. 1). Elsewhere irregularly distributed within the nucleus there may be a similar material giving a somewhat reticulated appearance to the nucleus. This material has the same tinctorial properties as the herpetic inclusions, but differs from the typical, well-developed herpetic inclusion morphologically.

The herpetic bodies stain best in our experience with eosin or erythrosin and stand out in good contrast to particles of chromatin in combinations of these stains with methylene blue, hematoxylin or other basic dyes. In certain forms to be described they are amphophilic, taking a fairly sharp blue in eosin-methylene blue preparations stained deeply with the basic dye following fixation in Helly's or Zenker's fluid.

In the form in which the inclusions most commonly occur they are readily recognizable and are associated with other nuclear or cytoplasmic changes which accentuate their prominence.

It is in ganglion and neuroglia cells of the central nervous system that it has been possible to study the inclusions to best advantage.

In motor ganglion cells they are readily distinguishable from the coagulated nucleoplasm of normal cells by their size and configuration. The material of which they are constituted greatly increases in amount and may form a compact crescent or ring about the nucleolus, and eventually it completely fills the intranuclear space which coincidently enlarges. In ganglion cells there is not the same tendency for fluid to accumulate within the nucleus as in other types of cells in herpetic lesions, and in consequence the intranuclear body may not be separated from the nuclear membrane by a clear zone. The nucleolus shows evidences of disintegration when the inclusions are well developed. It loses its symmetrical contour, becomes vacuolated or breaks up into irregular granules. The chromatin is collected about the nuclear membrane. The cytoplasm of such a ganglion cell shows chromatolysis partial or complete (Fig. 2).

In other cells of the rabbits in tissues acutely infected with the virus of herpes simplex, an intranuclear material with staining properties identical with that of herpetic inclusions in ganglion cells presents an even more conspicuous structure.

Intranuclear inclusions of this description have been found within cells from all three germinal layers infected with the virus. The nucleus tends to enlarge, chromatin particles collect about the nuclear membrane and the "inclusion" occupies the center of the nuclear space frequently separated from the nuclear membrane by a clear zone. Well-developed inclusions are often single; in earlier stages, however, there may be several irregular particles which seem later to coalesce to form one structure. The outline of the mass conforms in general to the shape of the nucleus, and may be quite irregu-

lar, round, oval or elongated. The more compact the inclusion is the more intense is its coloration by eosin. Usually the masses are not perfectly homogeneous and hyaline in structure but appear more or less honey-combed or roughened.

These are the forms of the inclusions which one first observes and which serve to distinguish the herpetic nature of a given lesion. But it is to be noted that the herpetic bodies are not static structures; they are progressive in their development. They tend to increase in size and eventually to fill the nuclear space. When the included material completely fills the affected nucleus a change may frequently be noted both in its tinctorial property and in its structure. The included mass in this stage takes a faintly basic stain in preparations in which smaller inclusions are stained a clear red with eosin, and they no longer appear rough and irregular in composition but seem to be composed of very minute bodies closely arranged and uniform in size. At this stage irregular granules of chromatin are distinguishable by their deep and sharp basophilic stain, their irregularity in size and their peripheral arrangement. In most tissues it is impossible to resolve clearly the small bodies constituting the inclusion, partly because of their minute size and compact arrangement, and partly because of the difficulty in staining the individual particles intensely. Even in this stage, nevertheless, the masses are readily recognizable as herpetic inclusions.

We have found in rabbits that the clearest preparations of the granules constituting the herpetic inclusion are to be obtained by inoculating herpetic virus directly into a corpus luteum of early pregnancy. At the end of 24 hours the corpus luteum cells contain well-developed inclusions and the ovary is rich in virus as has been determined by inoculating ground ovarian tissue into the brain or on the cornea of rabbits. The interstitial and corpus luteum cells show in great numbers the discrete form of inclusions separated by a clear zone from the nuclear membrane. In many of the larger cells of the corpus luteum, however, the nuclei enlarge to several times their normal diameter and become filled with extremely minute round or oval granules of uniform size sufficiently separate to be fairly well resolved. Irregular and deeply staining granules of chromatin are readily distinguishable from them. The structures constituting the herpetic inclusion are difficult to stain. They are amphophilic but can be brought out fairly sharply in blue in eosin-

methylene blue preparations deeply stained with the basic dye and not differentiated very far with colophonium acetone, dehydrated with acetone and mounted in cedar oil, or colored by a modified Giemsa's stain as recommended by Wolbach [10] but rendered slightly more alkaline.

The bodies thus demonstrated are smaller, more uniform in size and more numerous than the similar structures depicted by Lipschütz in preparations stained by Weigert's method after fixation in sublimate alcohol (Fig. 3).

It is not purposed here to attempt to identify these minute structures with the virus of herpes simplex. They have been described as they were found, constituting a phase in the development of the intranuclear inclusion of experimental herpetic infections in rabbits. For various reasons discussed elsewhere the conclusion has been reached that the virus of herpes proliferates within cells, and we hold with Lipschütz that the intranuclear body is a manifestation of the presence of the virus within the nucleus. It seems evident, however, that the material which constitutes the "inclusion" may partially at least be composed of coagulated nucleoplasm which may impart the acidophilic staining property of the inclusions. It is to be noted, however, that when the minute granulations are discrete enough to be recognized as such they stain faintly basophilically, whereas the precipitate from the nucleoplasm of normal cells is more acidophilic. They are to be regarded at present as elementary bodies taking part in the structure of the herpetic inclusions.

The nucleoplasm according to recent cytological investigations contains no preformed structure other than the nucleolus,[11] yet the herpetic inclusions may be seen in fresh unfixed preparations.[12] Consequently if there is a precipitation other than that due to fixation, it must be a result of changes attendant upon the infection. The indications are that the "inclusions" are present within the nuclei before the death of the cell, for they may be demonstrated typically within cells of the cornea and skin whose nuclei have divided to form the multinucleated giant cells which Unna described as constituting in part the "ballooning degeneration."

It remains to be proved whether or not these uniform granules which enter into the composition of herpetic intranuclear inclusions represent the virus itself, but in this respect the problem is the same as that presented by certain of the "inclusions" of other diseases,

which in the cell appear amorphous, but are really composed of minute particles of uniform size having the morphological appearance of microörganisms. The acidophilic cytoplasmic bodies of Geflügelpocke offer a striking analogy.

At the present time it is of most importance to recognize in these nuclear inclusions a change which is brought about specifically by the virus of herpes simplex, or closely related viruses. This fact to the writer is clear and it is believed will be readily recognizable if proper precautions are observed, and if early lesions of herpes are submitted to histological investigation. With the strains of virus which we have used the herpetic bodies may be studied to best advantage in lesions produced at the end of 24 hours after inoculation, wherever the site of inoculation or infection may be. The time element is a most important one if uniform results are to be expected, because the inclusions disappear rapidly in lesions caused by this virus, and in direct proportion active virus diminishes.

In studying herpetic lesions of the central nervous system it is frequently found that the initial lesion present at the entrance of a particular nerve may show few or no cells containing herpetic inclusions, while later lesions, as for instance, at the base of the cerebrum, will show innumerable ganglion cells thus affected. For this reason we cannot agree entirely with Parker [13] that human encephalitis lethargica is distinguishable from an herpetic infection because of the absence in the lesions of herpetic intranuclear bodies. In the event of an herpetic encephalitis of the human, the lesions to contain inclusions, presumably, judging from our experience with rabbits, would have to be very acute, and one would not expect to find them in every lesion but only in those representing latest extensions of the infection.

Recognition of the fact that an infection with the virus of herpes simplex is accompanied by these characteristic intranuclear inclusions in cells of local lesions is of importance not only in aiding to establish the herpetic nature of a given experimental lesion, but their presence serves to correlate histologically the lesion of three human diseases, herpes simplex, herpes zoster and varicella. Similar intranuclear inclusions ocur in cells of the cutaneous eruption of each of these infections and they are not found so far as we know in any other human diseases.

Corresponding to the histological similarity of the cutaneous le-

sions of these three diseases evidence is accumulating that they may be caused by very similar viruses. It has been shown that an experimental disease can be produced in rabbits and guinea pigs with the virus of herpes simplex quite analogous in many of its features to human herpes zoster,[14] and clinically there is considerable evidence that herpes zoster and chicken pox are closely related infections.

A better understanding of the so-called filterable viruses will undoubtedly throw much light upon the nature of the cellular inclusions which accompany the lesions of many of the acute infectious diseases of this nature, and in the meantime careful study of the bodies may lead us to a truer conception of the nature of this class of infectious agents, and to a closer critical analysis of the pathology of a most important group of diseases.

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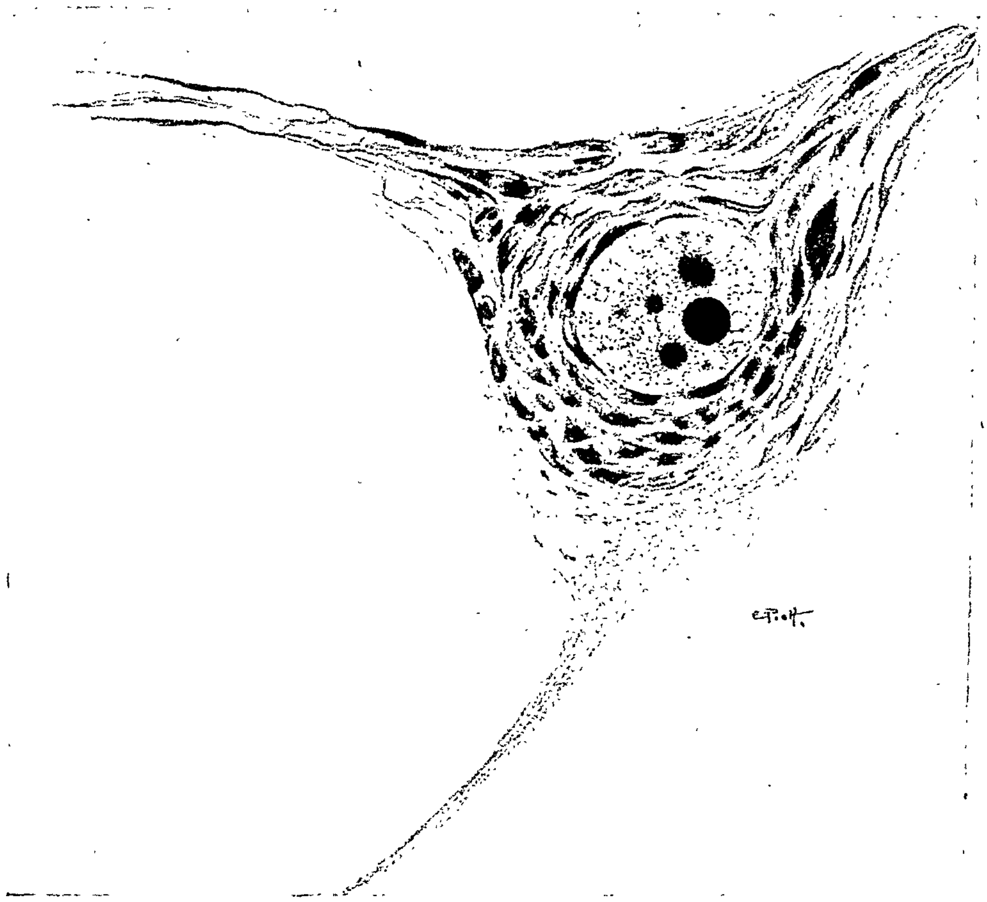
DESCRIPTION OF PLATE I

Drawings made from sections using No. 4 compensating ocular and 1.5 mm. objective.

Fig. 1. Normal multipolar motor ganglion cell from the motor nucleus of the fifth cranial nerve of a rabbit, showing dark circumscribed nucleolus about which are collections of material precipitated from the nucleoplasm. Nissl substance is abundant. Fixed in Helly's fluid, stained with hematoxylin and eosin.

Fig. 2. Multipolar ganglion cell from the motor nucleus of the fifth cranial nerve of a rabbit after inoculating the corresponding masseter muscle with virus of herpes simplex. The nucleus contains an irregular and vacuolated nucleolus partially surrounded by an irregular herpetic inclusion. Nissl substance has disappeared from the cytoplasm. Fixed in Helly's fluid. Stained with hematoxylin and eosin.

Fig. 3. Corpus luteum cell of a pregnant rabbit. The ovary was inoculated 24 hours before removal with virus of herpes simplex. The nucleus is enlarged and filled with minute structures uniform in size. The darker and somewhat larger bodies are particles of nuclear chromatin. There is an exudate of mononuclear leucocytes about the cell. Fixed in Helly's fluid. Stained with eosin and methylene blue.



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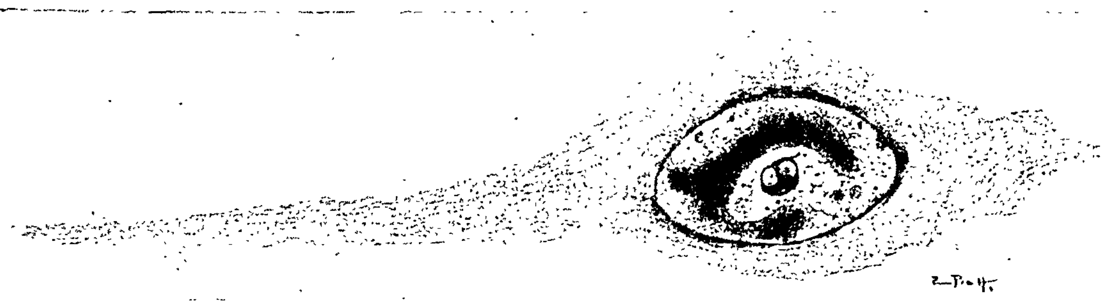

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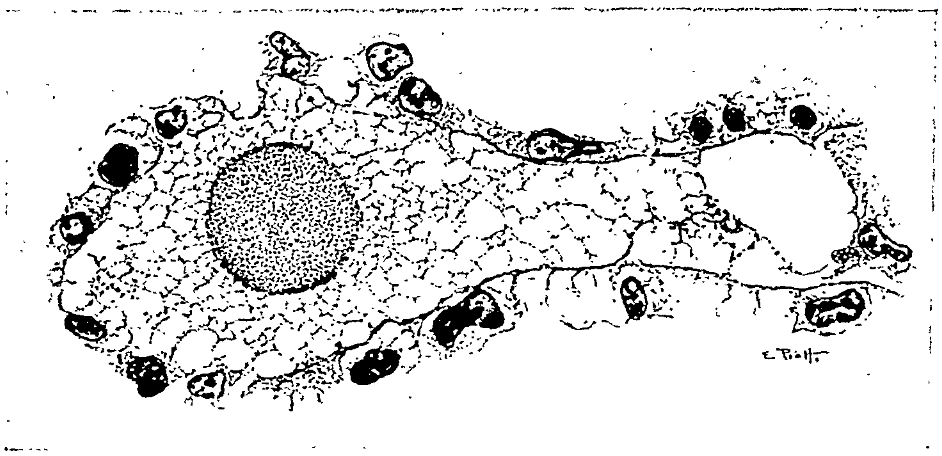

3

Goodpasture

Herpetic lesions of rabbits

THE AXIS-CYLINDERS OF PERIPHERAL NERVES AS PORTALS OF ENTRY TO THE CENTRAL NERVOUS SYSTEM FOR THE VIRUS OF HERPES SIMPLEX IN EXPERIMENTALLY INFECTED RABBITS *

Ernest W. Goodpasture

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A strongly neurotropic strain of the virus of herpes simplex derived from a herpetic vesicle on the lip of a patient with lobar pneumonia (virus M) has now been propagated through a series of rabbits by inoculations upon the scarified cornea for sixteen months and has, since its primary transfer to these animals, constantly induced a herpetic encephalitis when a uniform technique has been observed. With this strain it has been proved that the virus reaches the central nervous system through the medium of nerves supplying the peripheral areas primarily inoculated.[1,2] This mode of entry of the virus from the inoculated cornea into the pons has been confirmed by the experiments of Marinesco and Draganesco.[4] It has appeared that an initial local infection of cells at the site of inoculation is necessary for such an extension of the virus along the nerves into the central nervous system.

At the central origin or terminus of a nerve thus conveying the virus there is produced an acute local herpetic lesion which has been demonstrated grossly and microscopically.[3] The lesion is a destructive one and is characteristic for this infection, the cells involved presenting the acidophilic intranuclear inclusions of Lipschütz which have been shown to be a histological criterion of a herpetic lesion.[5]

The virus may be conveyed along sensory, motor or sympathetic nerves depending upon the innervation of the peripheral site inoculated, as for example, following an infection of the cornea an acute herpetic encephalitis results involving the sensory root of the fifth cranial nerve in the pons and medulla on the side inoculated; following an injection of the virus into the muscles of a hind leg an acute myelitis is produced in the lumbar portion of the spinal cord; or if the virus is inoculated into an adrenal gland or an ovary an

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acute myelitis follows, striking the level of the spinal cord where sympathetic fibers from these organs enter. Inoculations into many other localities have given abundant confirmation of a neural transmission of the virus from a peripheral focus of infection and its ready transplantation upon the brain or spinal cord at its point of entrance where conditions for its growth and extension are exceptionally favorable.[2]

In a previous study of the neural transmission of the virus of herpes from a peripheral focus of infection to the central nervous system the opinion was expressed that the virus passed along axis-cylinders rather than by way of perineural spaces. This conclusion was arrived at from a consideration of several points. The virus seemed to grow within cells rather than in the tissue fluids. We were unable to transmit a herpetic infection by injecting the blood of an infected rabbit into a normal susceptible rabbit, nor were we able to cause an encephalitis by inoculating the virus into loose connective tissue, care being taken to avoid an infection of the epidermis about the wound. The presence of acidophilic intranuclear inclusions of Lipschütz[6] was regarded as representing an intracellular growth of the virus. The lesions produced within the central nervous system following a peripheral infection were proved to be directly associated with the nerve from the periphery, and the fibers of this nerve evidently directed the course of an extending infection within the brain or spinal cord after the virus had gained an entrance. The axis-cylinders being protoplasmic processes of ganglion cells, it was thought probable that the virus actually invades and grows along them.[4] However, the animals in our previous experiments were permitted to live usually for several days after they had shown evidences of cerebral disease and on histological examination the herpetic lesions had extended considerably from the point of entrance into the brain and there was always an associated local meningitis at the point of entry, consequently it was felt that the possibility of a perineural dissemination of the virus could not be entirely excluded.

The following experiments, it is believed, present convincing evidence that the virus does extend along the axis-cylinders and not, as maintained by Marinesco and Draganesco, through perineural spaces, and that it invades the central nervous system from a peripheral focus of infection by propagating itself along these cellu-

lar processes which for the most part in their extracranial courses are protected from other tissues by their myelin sheaths and the sheaths of Schwann.

Satisfactory conditions for the experiments were found in the anatomical structure of the fifth cranial nerve, and the situation of its motor nucleus in the depths of the pons and separate from the large descending crescentic bundle of its sensory fibers.

The fifth motor nerve throughout most of its extent lies in close proximity with sensory fibers. After it passes through the Gasserian ganglion, which folds partially about it on either side from above, and branches downward, it is accompanied for a considerable distance by the inferior maxillary division of the nerve. Between sensory and motor fibers there are scarcely enough delicate fibrils of connective tissue to make the limits of each group distinguishable. The two types of fibers are readily distinguishable in microscopic sections, however, because the axis-cylinders of the motor nerve are larger and are surrounded by a more compact-appearing myelin sheath. This greater diameter of the motor nerve fiber is quite marked at the proximal end. There is also a greater number of neurolemma cells in the sensory portion of the nerve.

The sensory and motor divisions of the fifth nerve enter the brain together, the latter having a ventral position; and a short distance from the brain they both suddenly lose their sheaths of Schwann in a sharp transverse plane which is common to both. Central to this point the myelin sheaths appear much looser and less substantial than peripherally, and neuroglia cells are situated here and there between the fibers, most numerously in the sensory division, yet the motor nerve continues to have the appearance of being more substantially protected by myelin.

On entering the pons the motor and sensory divisions separate, the sensory group forming a crescentic tract which extends caudally near the periphery traversing the medulla, while the motor division enters the pons as a compact group of fibers, extends dorsally and medially, the major portion finally arching caudally to end in a circumscribed group of large multipolar motor ganglion cells in which they have their origin and which constitute the motor nucleus. A smaller group of fibers continues dorsally to end in the mesencephalic or accessory motor nucleus situated laterally at the angle of the floor of the fourth ventricle. In the proximal third of their in-

tracerebral course the myelin sheaths are more delicate, and they gradually disappear as the nerves penetrate the nucleus.

The presence of the myelin protective sheath is of much importance in interpreting the results of the following experiments. It has previously been shown that following an inoculation of the cornea on one side there may be no certain lesion of the extradural portion of the sensory division of the fifth cranial nerve which innervates the cornea and along which the virus passes to the brain, but there is constantly an acute herpetic lesion immediately central to the transverse plane where the sheath of Schwann disappears, and from there it may extend throughout the intracerebral course of the sensory tract. It was believed this sharp delimitation of the lesion was to be explained by the fact that in the proximal portion of the nerve the axis-cylinders are less well protected by a myelin sheath permitting the virus to escape from infected axis-cylinders and to enter the susceptible neuroglia cells which surround them. But in the case of a sensory nerve it was not possible to determine accurately whether the virus followed the axis-cylinder to its termination, or whether liberated into perineural spaces on its entrance into the cerebral tissue because of less protection by myelin sheaths it was only directed in its spread by the bundle of fibers in which it happened to be situated. The conditions were suspected to be different, however, with the motor division of the fifth cranial nerve, having its central origin in large ganglion cells deeply situated within cerebral tissue; for if, as was assumed, the virus traversed axis-cylinders then the motor ganglion cells should show evidences of infection very early, and since the myelin sheaths of the intracerebral portion of the fifth cranial nerve are apparently more substantial than those of the corresponding sensory division, it was believed that the chances of escape of virus along the intracerebral extent of the nerve would be less. If the virus passed merely along perineural spaces on the other hand, one would expect it to be liberated at the entrance of the nerve into the brain, giving rise to an infection of neuroglia cells at this point with an accompanying local meningitis and herpetic lesions perhaps throughout its extent, but naturally involving neuroglia along the nerve and in the nucleus before the ganglion cells were affected, the latter perhaps escaping altogether in early stages.

To test this hypothesis it was decided to select as a point of attack the motor division of the fifth cranial nerve by inoculating the virus

of herpes directly into the masseter muscle and killing the rabbit at the first elevation of temperature, which we believe is the earliest indication of a herpetic encephalitis. By means of serial sections through a proper region of the pons the complete intracranial course of this nerve could be studied and the progress of the infection observed.

By this procedure it has been possible to show that following inoculation of the masseter muscle on the right side, intracerebral herpetic lesions are first demonstrable within the motor nucleus of the fifth cranial nerve, and the motor ganglion cells are apparently the first to be attacked as was anticipated, the medullated fibers passing through a wide extent of susceptible tissue without evidences of neuroglia infection, which would hardly be possible were the virus outside the medullary sheaths. Under these circumstances, there may be no evidence of a neuritis in the extradural portions of the motor nerve.

Material for inoculating the masseter muscle was obtained by inoculating the cornea of a rabbit with 24 or 48 hour virus (from an infected cornea) and at the end of 24 hours, anesthetizing the animal, scraping the cornea lightly with a sterile scalpel, and taking up the material from the conjunctival sac by washing off the cornea with 1 or 2 c.c. of sterile salt solution and aspirating with a syringe. From one half to the entire amount of material thus removed, suspended in about 1 c.c. of salt solution, was used for a single inoculation. In inoculating the virus into the muscle the material has been injected into several places among the muscle fibers without removing the needle, but by changing its depth and direction. The animals were then killed by etherization and exsanguination at the first distinct elevation of rectal temperature.

The brain was immediately fixed by injecting Helly's fluid under considerable pressure from a syringe into each carotid artery. It was then removed and placed in the same fixing fluid for 24 hours. Blocks were cut from various portions including the entire brain in cross section. In no instance in the following experiments was a cerebral herpetic lesion found beyond the motor division of the fifth cranial nerve and its nucleus on the side inoculated, excepting in Experiment 7, in which the rabbit was permitted to die of the infection. Here the infection had extended throughout the medulla and to the base of the cerebrum. Consequently in most of the ex-

periments described below only sections including the fifth motor nerve and its central distribution are recorded.

The following are typical experiments in most of which serial sections were studied.

Experiment 1.

R. 148–24.

1/9/24. Adult rabbit. Injected 0.5 c.c. suspension of pus and scrapings from right eye of R. 147 (inoculated 36 hours previously) into multiple places in right masseter muscle.

1/11. Temp. 103° F.

1/13. Temp. 103°.

1/14. Temp. 105.8°. Etherized. Brain, right fifth nerve and right masseter muscle saved for section.

Microscopic Examination. Serial sections through the pons at the level of the fifth motor root and nuclei. Microscopic sections show no meningitis at the entrance of the nerve and no lesion along the course of the nerve to the motor nucleus. Within this nucleus on the right side there is in places a slight cellular infiltration with phagocytic mononuclear cells and here and there are neuroglia cell nuclei containing typical herpetic intranuclear bodies. The main lesion, however, is to be found in several motor ganglion cells. Some of these show typical intranuclear bodies, sometimes with nucleoli intact, and chromatolysis. Others are necrotic and shrunken. There is no neurophagocytosis. The motor nucleus on the opposite side is quite normal and there is no lesion in the pons other than the foci about ganglion cells within the fifth motor nucleus. The sensory bundle of the right fifth nerve shows no lesion. In one section after the motor bundle has reached the level of the motor nucleus and is turning inward toward it, two neuroglia cell nuclei are found between nerve fibers containing intranuclear inclusions. There is no other evidence of infection here. Similar cells are found in other sections, but always at the central end of the nerve.

Serial sections through the right fifth motor nerve including the entire portion from the dura through the Gasserian ganglion and beyond, a total extent of over 1 cm., show no lesion.

This case shows that the virus may be transmitted along the motor nerve without evidence of its presence until the terminal third of its intracerebral portion is reached, that is, on a level with the motor nucleus. Here the myelin sheath is thin and axis-cylinders are poorly protected so that virus within axis-cylinders may escape and infect neuroglia cells with which it comes in contact.

Experiment 2.

R. 133. Adult rabbit.

12/21/23. Right masseter muscle inoculated in several places with washings from right eye of R. 131 (24 hour virus).

12/24. Temp. 104° F. Etherized. Autopsy negative.

Pons at entrance of fifth cranial nerves. There is no cellular exudate in the meninges about the entrance of the fifth nerve on the right or elsewhere. The motor bundle can be followed throughout its intracerebral extent and shows no lesion nor the presence of any intranuclear bodies in neuroglia cells. In the

upper pole of the right motor nucleus, however, there is a moderate general infiltration with mononuclear phagocytes. In small foci they are collected about necrotic ganglion cells. Many neuroglia cells immediately about the dead cells contain typical intranuclear herpetic inclusions. No herpetic lesion was observed elsewhere in the brain.

Right fifth motor nerve. Serial sections through the right fifth nerve were made, including that portion from a point several millimeters distal to the Gasserian ganglion to a point central to the plane intradurally where the sheaths of Schwann disappear, a total distance of 1.5 cm. Throughout this entire extent no lesion of the nerve was observed and no intranuclear herpetic inclusions were found in the cells of the neurolemma.

In this case, therefore, the only herpetic lesion found in the distribution of the nerve was at its central termination in the motor nucleus, and here as has been uniformly the case ganglion cells as well as neuroglia were destroyed by the herpetic infection.

Experiment 3.

R. 224. Adult non-pregnant female rabbit.

3/21/24. Right masseter muscle injected with virus in saline washings from left eye of R. 219 (inoculated 24 hours previously).

3/23/24. Temp. 102.8° F.

3/24/24. Temp. 103°.

3/25/24. Temp. 103°.

3/26/24. Temp. 105.8°. There is edema and congestion of the right eye and lacrimation. No pus is present. Cornea is clear. No turning of the head. Etherized.

Microscopic Description.

Serial sections of the pons at the entrance of and including the central distribution of the motor division of the fifth cranial nerves. At the entrance of the right fifth cranial nerve and over the base of the pons there is a slight exudate of mononuclear cells in the meninges. No lesion of the motor nerve is observed until a point half way in its intracerebral course is reached. Here there is a small focus of mononuclear phagocytes, but no intranuclear bodies are found. Central to this point there are small foci of such cells but in inconspicuous numbers. No relatively significant herpetic lesion is found until the nerve enters the motor nucleus. In the right motor nucleus there is an abundant mononuclear phagocytic cell exudate throughout its entire area and many necrotic ganglion cells are found, some of them surrounded by a small group of these cells. An occasional polymorphonuclear leucocyte is observed. Notwithstanding the abundant cellular exudate, only an occasional neuroglia cell contains an intranuclear inclusion. The exudate is apparently due entirely to the destruction of motor ganglion cells. Some of these are completely destroyed, others show typical herpetic inclusions and are undergoing disintegration.

Right fifth cranial nerve. Serial sections were made through this nerve including the motor bundle from a point distal to the Gasserian ganglion to near its entrance into the brain, a length of approximately 1 cm. Throughout its course one finds here and there small groups of mononuclear phagocytic cells situated among the fibers. These foci are not numerous but are very definite.

Careful study of these lesions proves each of them to be an herpetic neuritis. Each focus of cells is collected about an individual motor fiber which can be

traced through many sections. The entire extent of the fiber is not involved, the exudate occurring only at certain points. Three or four fibers presenting a surrounding exudate may be found in a low power field including the entire breadth of the nerve, and there are a few mononuclear phagocytes about small blood vessels in the neighborhood of the lesion. The earliest changes noted in such lesions are in the nucleus of a cell of Schwann's sheath. The nuclear chromatin is granular and thin, lying peripherally along the nuclear membrane. The interior of the nucleus is almost filled by a homogeneous or finely granular eosin-staining material characteristic of the herpetic intranuclear inclusions found so frequently elsewhere in acute herpetic lesions. The axis-cylinder immediately beneath such a cell takes a deeper eosin stain than others and it appears homogeneous or very finely granular and vacuolated. In more advanced lesions there is an accumulation of amoeboid mononuclear phagocytes arranged about the individual nerve fiber, and these cells seem in places to have penetrated the myelin sheath and to be phagocyting particles of the axis-cylinder, as several were found in such a situation containing globules of pink staining hyaline material within their cytoplasm. The nerves have not been stained for fat and no certain changes in the myelin sheaths have been demonstrated.

The relation of the cells of the neurolemma which contain herpetic inclusions to the lesions is such as to leave little doubt that the cellular exudate occurs only about those fibers, and at those points along an individual fiber, where a cell of Schwann is infected with the virus of herpes.

Experiment 4.

R. 275. Adult rabbit.

4/26. Right masseter muscle injected with 1 c.c. of a salt solution suspension of material from both eyes of R. 274 (inoculated 24 hours previously).

4/29. Temp. 102.6° F.

4/30. Temp. 102.7°.

5/1. Temp. 102.5°.

5/2. Temp. 102.8°.

5/3. Temp. 104°. Etherized. Autopsy negative.

Microscopic examination of the brain. Paraffin blocks were made from various cross sections of the brain and spinal cord. Sections showed no evidence of herpes other than in the pons as described below. Serial sections were made through the intracerebral distribution of the fifth cranial nerves and motor nuclei. In the intradural portion of the motor root of the fifth nerve there are small foci of mononuclear cells here and there, but no intranuclear bodies are found. There is a sharp change at the point where the nerve loses its sheath of Schwann. Here there is an abundant exudate of mononuclear leucocytes and some polymorphonuclear leucocytes. Many cells are found in foci of exudate which contain typical herpetic intranuclear bodies. There is about the nerve here a cellular exudate in the meninges consisting of mononuclear leucocytes. A few neighboring blood vessels in the peripheral brain tissue are surrounded by a mantle of mononuclear cells.

The acute herpetic lesion of the nerve extends irregularly and with diminishing intensity from the line where the sheath of Schwann is lost until the nerve enters the brain. From this point no herpetic lesion is found in the nerve as it courses through the brain until the fifth motor nucleus is reached. In the nucleus and limited by its confines there is a general moderate infiltration with

mononuclear leucocytes, which in several small foci are grouped together. There is also a slight perivascular infiltration. Here and there neuroglia cells contain the herpetic inclusions in their nuclei, and necrotic ganglion cells are found showing chromatolysis and intranuclear herpetic inclusions. Grouped about such cells is a margin of mononuclear leucocytes.

This animal lived two days longer than the other acute cases after inoculating the masseter muscle, and there is an acute herpetic neuritis of the intradural portion of the motor nerve. Proximal to this point, however, the nerve presents no lesion, but there is an extensive circumscribed herpetic lesion of the fifth motor nucleus.

Experiment 5.

R. 276. Adult female.

4/26/24. Right masseter muscle inoculated by injecting salt solution suspension of material from both corneas of R. 274 (inoculated 24 hours previously).
4/29. Temp. 102.4° F.
4/30. Temp. 102.4°.
5/1. Temp. 104.5°. Etherized. Autopsy negative except for early pregnancy.

Microscopic Sections. Serial sections were made including the entire intracerebral extent of the motor root and nucleus of the fifth cranial nerve. In sections through the anterior pole of the nucleus there is a moderate infiltration with endothelial leucocytes, which in one or two places are grouped in small aggregations. There are no intranuclear bodies found here in the neuroglia cells. The cellular exudate is strictly limited to the nucleus and there are several degenerated ganglion cells, some of which show typical intranuclear bodies. The necrotic ganglion cells are the center of an accumulation of mononuclear leucocytes. There is slight perivascular infiltration in the neighborhood of necrotic ganglion cells.

In the terminal third of the motor root there is a small group of cells among which are one or two neuroglia cells containing herpetic inclusions. There is a slight increase in the number of mononuclear leucocytes in the meninges over the region of entrance of the fifth nerve, unassociated with any lesion of the nerve at this place. This may be a result of the herpetic infection or possibly of an associated spontaneous encephalitis of rabbits, as it is present in equal proportion at other places in the meninges.

In this case the herpetic infection in the brain as determined by the presence of the intranuclear bodies is limited to the anterior half of the fifth motor nucleus and to a very small focus in the terminal third of the intracerebral portion of the fifth motor nerve root. The left motor nucleus is normal.

Experiment 6.

R. 277. Adult rabbit.

4/26. Right masseter muscle injected with salt solution suspension of material from both eyes of R. 276 (24 hour virus).
4/29. Temp. 104° F.
4/30. Temp. 103.7°.
5/1. Temp. 106°. Etherized. Autopsy negative.

Microscopic Sections. Serial sections through fifth motor root and nucleus show no lesion either in the fifth nerve or in the nucleus. There is no indication of herpetic encephalitis. The temperature reaction of this animal was a little different from the others in that it was above normal (104° F.) on the third day and was extremely high on the fifth (106° F.). No explanation is offered for the failure to find herpetic encephalitis.

Experiment 7.

R. 449. Adult rabbit.

6/9/23. Right masseter muscle inoculated with 24 hour herpes virus from eyes of R. 439 and R. 435.

6/14. Dead. Autopsy negative.

Microscopic sections through fifth motor nucleus. On the right side there is an extensive acute meningitis at the entrance of the motor root into the brain. Brain tissue is destroyed immediately about the bundle of fibers as it enters, and is replaced by inflammatory cells. The meningeal exudate is composed both of polymorphonuclear and mononuclear cells, the latter predominating. There is also considerable fibrin.

The bundle of motor fibers as it passes through to the interior of the medulla is greatly swollen containing large spherical spaces. It appears to be very edematous. At foci along its course are small collections of mononuclear and polymorphonuclear leucocytes in the neighborhood of which are cells, apparently neuroglial, containing intranuclear bodies. To either side within the surrounding brain tissue there is a moderate mononuclear infiltration, and a perivascular infiltration with similar cells, but no intranuclear bodies are found here.

Serial sections were not made but sections through the central portion of the nucleus show a most intense change. Every ganglion cell is necrotic, many completely replaced by polymorphonuclear leucocytes, others contain typical intranuclear bodies, others are faded and vacuolated. There is a general edema and cellular infiltration with polymorphonuclear and mononuclear leucocytes, the latter predominating, but the former phagocyting the dead cells. Numerous neuroglia cells contain intranuclear bodies. Neuroglia cell nuclei containing intranuclear bodies and an accompanying cellular infiltration extend in a zone about the motor nucleus. In bundles of transverse fibers, extending across the midline on a level with the right nucleus, there are small foci of leucocytes with a few neuroglia cells containing intranuclear bodies. These can be followed to the opposite fifth motor nucleus on the left. Here there is also an extensive herpetic infection, but evidently more recent than on the right side. There is scarcely any cellular infiltration, but a great many of the neuroglia cells and most of the ganglion cells show intranuclear bodies of various stages of development, and chromatolysis. There is no neurophagocytosis. The infection as thus indicated is limited to this nucleus and to certain points along the corresponding bundle of motor fibers. The infection on the left side is fairly sharply limited to the motor nucleus though about the periphery there are small foci of infection in other groups of cells. There is no acute meningitis on this side and the cerebellar and pontine tissue elsewhere in the section seems to be free of lesions.

Medulla. Sections through the medulla show the infection has extended throughout on either side near the center of each hemisegment of the medulla. In this area on each side are numerous neuroglia cells containing intranuclear

bodies and some cellular infiltration and perivascular infiltration. The sensory tracts show no change.

The necessity of obtaining for this study the brain of an infected rabbit immediately after the onset of fever is illustrated by the rapid and diffuse spreading of the infection in the pons and medulla in Experiment 7. At such a stage it is not possible to determine with certainty the earliest lesion. It is of interest to observe in this case that an extension of the infection directly across the midline of the pons from the right fifth motor nucleus to the corresponding nucleus on the left. It seems possible that this extension took place along nerve fibers which may associate the two nuclei.

There is no adequate explanation at hand for the negative result in Experiment 6. The fact that the temperature became slightly elevated on the third day makes it seem possible that the febrile reaction resulted from the initial infection in the masseter muscle. If this is true, it is unique in our experience. The possibility of an intercurrent infection was not excluded.

Experiment 4 differs from the first three in that there was no elevation of temperature until the seventh day after injecting the masseter muscle, and in the fact that there is in addition to the extensive lesion in the motor nucleus on the inoculated side a neuritis especially marked just central to the plane at which the sheath of Schwann is lost. This is similar to the lesion which occurs in the intradural portion of the sensory division of the fifth cranial nerve following inoculation of the cornea. It has been shown in previous experiments that following inoculation of herpetic virus into muscles of the hind leg a neuritis of the sciatic nerve may occur in case the animal lives for several days after the inoculation. Apparently the virus may destroy a nerve fiber, penetrate the myelin sheath, and become liberated, producing a local lesion within the nerve under certain circumstances which will be considered later. Such a neuritis is illustrated in Experiment 5. In these experiments, however, the spread of the virus to the motor nucleus was evidently within the myelin sheath as there was a long interval between the motor nuclei and the immediate intradural lesion of Experiment 4 and the extradural lesions of Experiment 5, in which the nerve bundles showed no evidence of herpetic infection either by the presence of intranuclear bodies within neuroglia cells or by a cellular exudate, yet within the confines of the nuclei there was in each an extensive infection with

destruction of ganglion cells, but no greater in extent than in cases showing no neuritis. Experiment 4 may serve as a control for 1 and 2 in that it demonstrates the susceptibility of the tissue about the proximal portion of this nerve if virus escapes from the myelin sheath.

In Experiments 1 and 2 the conditions of the test were satisfactory and the result fulfilled expectations based upon the hypothesis that the virus traverses the nerve along axis-cylinders. In these animals serial sections through the fifth cranial nerve distal and proximal to and including the Gasserian ganglion show no evidence of a herpetic lesion. Minor changes in axis-cylinders could not be detected by the histologic methods used. There were, however, no intranuclear bodies or cellular infiltration throughout the extradural portion of the motor division of the nerves. In the intradural portion proximal to the plane of Schwann's sheath there was likewise no evidence of a lesion. Almost throughout its entire extradural extent the motor nerve is in close proximity to sensory fibers yet there was no escape of virus into the sensory nerve sufficient to produce a lesion in the proximal termination of its fibers where the tissue is very susceptible to the virus.

Throughout the intracerebral course of the nerves in these two experiments there was no evidence of a lesion until the terminal third of the motor bundle was reached just before the fibers disperse to enter the nucleus. Here the myelin sheaths are thin and diminishing in size so that virus contained within them might be permitted to escape, infecting as it did so neighboring neuroglia cells as indicated by the intranuclear bodies of Lipschütz. Had the virus been present in perineural spaces or had it escaped from the myelin sheaths peripheral to this point undoubtedly a local herpetic lesion would have resulted as was demonstrated in Experiment 4. But the few neuroglia cells infected in the terminal portion of the nerve are insignificant compared with the extent of injury and reaction within the nucleus itself. In every case where herpetic encephalitis has followed inoculation into the masseter muscle ganglion cells within the corresponding nucleus have been shown to be necrotic or to contain herpetic intranuclear inclusions, and in these two cases it seems evident that the virus goes directly to the ganglion cell by means of its axis-cylinder. The lesion is not confined to the point of entrance of the fibers into the nucleus, but ganglion cells here and there are

picked out showing in earliest stages intranuclear inclusions and chromatolysis often without an apparent infection of neuroglia cells immediately about. In both cases, however, as was to be expected, neuroglia cells in certain places within the nucleus do contain herpetic inclusions. In each case there was a mild exudation of large phagocytic mononuclear cells with indented nuclei scattered irregularly (Experiment 1) or in addition occurring in groups (Experiment 2). There were no polymorphonuclear leucocytes and no hemorrhage. About small blood vessels within the nucleus were gathered a few mononuclear leucocytes of the type present in the tissue. While many neuroglia cells in each instance contained intranuclear inclusions, none was found in a necrotic state. On the other hand, several necrotic ganglion cells were observed in each case.

The number of ganglion cells infected conformed to what one might expect from an extension of the infection along axis-cylinders. In inoculating the masseter muscle virus was injected into several places, yet with the best of success only a relatively few nerve endings probably were infected, the great majority of those in this muscle remaining out of contact with the virus. Microscopic sections of an inoculated muscle show only scattered foci of inflammation. Under these circumstances the virus could be carried only by relatively few axis-cylinders. If the virus were taken up by perineural fluid and transplanted passively to the brain, it would in all probability regularly infect the cells of Schwann's sheath before it reached the brain, and then of necessity would come in contact with susceptible neuroglia cells, producing its most intense effect as the nerve entered the brain perhaps without reaching the nucleus at all or only later. Were the virus growing in perineural fluid and extending inward by propagating itself, a similar result though more intense would be expected. The passage of the virus directly from the inoculated muscle to the motor nucleus without evidence of a lesion until the nucleus is reached and there picking out ganglion cells almost specifically leaves little room for doubt that the mode of transit is by way of axis-cylinders. It is difficult to conceive of a passive transportation of the virus within axis-cylinders and considerable evidence is at hand that herpetic virus may proliferate within cells. It is consequently believed that the virus grows within the axis-cylinders until it reaches the body of the cell itself, which it destroys, usually penetrating and proliferating within the nucleus

before death of the cell occurs. The liberated virus then spreads to the surrounding neuroglia.

Various stages in the disintegration of ganglion cells may be observed. The earliest is the appearance of irregular masses or a single large mass of acidophilic material within the nucleus associated with an irregular nucleolus. It is evident that the acidophilic material arises within the nucleoplasm and is not derived from the nucleolus. In the earliest stages in which these herpetic inclusions were recognized there was also an obvious chromatolysis. Later the entire nucleus may be filled with a finely granular acidophilic mass. The nucleolus disintegrates and disappears; chromatin particles appear about the nuclear membrane; chromatolysis is completed. The cell thus disintegrates and is phagocyted by polymorphonuclear or mononuclear leucocytes. Cells once entered by the virus as indicated by nuclear inclusions do not recover but invariably undergo necrosis. In early lesions, as illustrated in Experiments 1, 2 and 3, neurophagocytosis of ganglion cells was not observed. In Experiment 7, most of the ganglion cells on the right side were being thus removed, this animal having lived longer, and the lesions were considerably more advanced.

Herpetic Neuritis. It has previously been shown that a neuritis of the sciatic nerve may follow inoculation of herpetic virus into muscles of the hind leg of a rabbit, though we were not able in certain cases to demonstrate a neuritis of the sensory portion of the fifth cranial nerve following inoculation of the cornea.

Marinesco and Draganesco [4] have demonstrated an inflammatory reaction within and about the ciliary nerves and in the ciliary and Gasserian ganglia following herpetic keratitis, and have assumed that herpetic virus enters the central nervous system by propagating itself from a peripheral focus of infection through lymphatic and perivascular spaces of the nerve to the brain.

Experiments presented above demonstrate that the virus may enter the motor nucleus of the fifth cranial nerve following inoculation of a masseter muscle leaving no discovered trace in the motor nerve of its passage. Yet as shown in Experiments 4 and 5, a herpetic neuritis may occur under the conditions of this experiment and a study of the neural lesions in serial sections of the nerve in Experiment 5 has shown clearly how they may occur; and the pathogenesis of the lesions contributes further evidence in our opinion

that the herpetic virus does not propagate itself along perineural and perivascular spaces to enter the brain, but invades the brain through axis-cylinders.

The earliest stage of herpetic neuritis is to be found in a cell of Schwann's sheath, and in the underlying axis-cylinder. Before there is a cellular infiltration about the nerve fiber, the nucleus of a neurolemma cell may be found to present the changes characteristic of a herpetic infection. The chromatin becomes concentrated in a thin broken line peripherally upon the nuclear membrane, while the interior of the nucleus is filled almost completely by a homogeneous or very finely granular eosin staining material. The underlying axis-cylinder appears very faintly granular, vacuolated, and takes a light eosin stain. Sometimes it appears fragmented. Following these changes mononuclear phagocytic cells accumulate about the nerve fiber and may penetrate to the interior of the fiber, and seem to phagocyte particles of the disintegrating axis-cylinder. There is at the same time an accumulation of similar cells about neighboring small blood vessels from which the phagocytes possibly migrate. Occasionally a polymorphonuclear leucocyte is found in the exudate.

The evidences of inflammation of the nerve in the form of a cellular exudate depend upon an infection of cells of the neurolemma by the virus of herpes, and the local injury produced as a result.

The fact that cells of the neurolemma are thus susceptible to infection by the virus of herpes immediately suggests a possible pathway for the virus to propagate itself intracellularly from one neurolemma cell to another from the periphery to the central nervous system without necessarily invading axis-cylinders, for these cells are possibly contiguous from the periphery to the intradural space. This would seem a more acceptable hypothesis than that the virus propagates itself only in perineural spaces. But histological evidence does not support such a conclusion. A study of the serial sections of the motor nerves from Experiments 1 and 2 has revealed no indication of herpetic infection of neurolemma cells, or of inflammation of any kind, yet virus traversed the nerve and through it entered the motor nucleus, destroying ganglion cells.

In the serial sections of the nerve from Experiment 5, inflammatory foci and cells of Schwann's sheath containing intranuclear herpetic bodies are not numerous and occur focally along single nerve

fibers. They remain local, and wide areas of nerve in its longitudinal axis may be free from evidences of injury.

In the event that herpetic virus propagated itself through perineural lymph spaces, it is hardly possible that cells of the neurolemma would escape infection in any instance, and if such infection did occur, there would regularly result an inflammatory focus.

Another explanation for infection of the cells of Schwann's sheath and the resulting neuritis in Experiments 4 and 5 is that virus from an infected axis-cylinder along which it was propagating itself entered these cells from within the myelin sheath to which the neurolemma cells are closely applied. That this occurred is indicated by the fact that axis-cylinders lying beneath the neurolemma cells, which contain herpetic inclusions, present evidences of disintegration, and it is certain that the center of origin of a focus of herpetic neuritis is an individual nerve fiber. It is about such a fiber that inflammatory cells accumulate and following its degeneration phagocyte its remains. Perivascular infiltration and the appearance of inflammatory cells between neural fibers is a secondary reaction to the injury resulting from an herpetic infection within the fiber and of one or more overlying cells of Schwann.

The possibility of an ascending herpetic neuritis resulting from a succession of focal infections of the susceptible cells of the neurolemma, which might reach the brain with a liberation there of the virus, is recognized and can be excluded from a particular case only in the absence of evidences of neuritis as demonstrated in Experiments 1 and 2. It is probable that such a mode of propagation of the virus plays no part in the production of herpetic encephalitis, for herpetic lesions outside nervous tissue do not tend to spread far to infect neighboring cells, but, as frequently demonstrated in the cornea, tend to remain localized in small areas primarily inoculated.

Conclusions

1. Following inoculation of the virus of herpes febrilis into the right masseter muscle, an herpetic encephalitis usually occurs within five to seven days as is indicated by an elevation of body temperature.

2. There is uniformly demonstrable a herpetic lesion in the brain of such animals within the motor nucleus of the right fifth cranial nerve.

3. In early cases no lesion of the corresponding motor nerve may be demonstrated by serial sections until the nucleus is reached.

4. It has been shown that the nerve, especially its intradural portion, is susceptible to infection if the herpetic virus comes in contact with surrounding neurolemma or neuroglia cells.

5. The experiments presented, it is believed, prove that an axis-cylinder transmission of the virus of herpes simplex from a peripheral site of inoculation to the central nervous system occurs and that the virus may be prevented from invading surrounding tissue along the course of the nerve by the myelin sheaths. It seems most probable that the virus grows within the axis-cylinder propagating itself in this way to its central termination, infecting there the highly susceptible cerebral tissue and spreading within the brain in a similar fashion to more distant areas.

6. The possibility of an ascending herpetic neuritis by a propagation of the virus through cells of the neurolemma, which are shown to be susceptible to infection, is recognized, and can only be excluded in the absence of evidences of inflammation and of herpetic inclusions in the course of a particular nerve through which virus has entered the central nervous system.

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DESCRIPTION OF PLATES II–IV

Plate II

Fig. 1. High power of the right motor nucleus of R. 148 showing destruction of ganglion cells, and a cellular exudate.

Plate III

Fig. 2. R. 133. High power of an area in the right motor nucleus showing a necrotic ganglion cell in the center and a normal ganglion cell to the left. The nucleus of the necrotic ganglion cell contains fragmented chromatin and is otherwise filled with eosin-staining material characteristic of herpetic inclusion. There is complete chromatolysis.

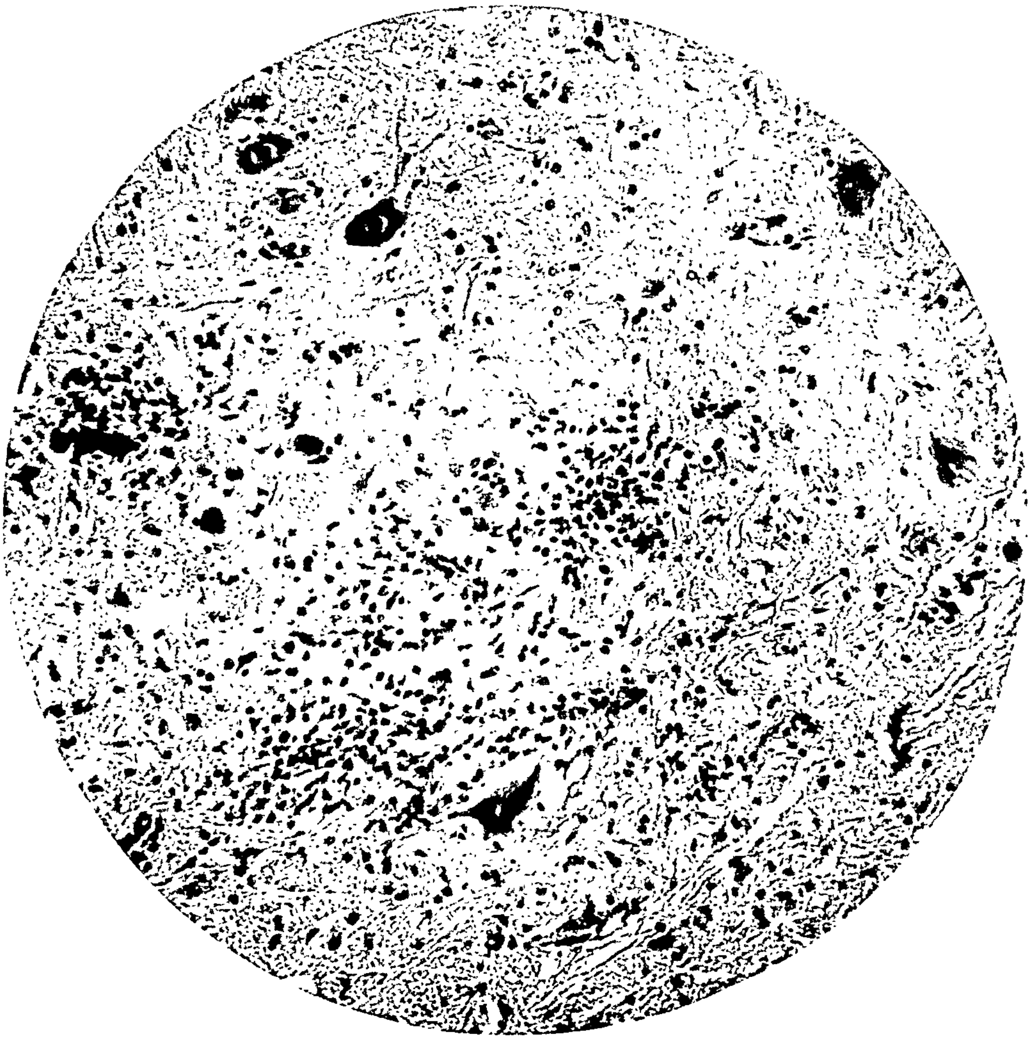
Fig. 3. Intranuclear bodies in neuroglia cells within the fifth motor nucleus.

Fig. 4. R. 449. Necrotic motor ganglion cell and phagocytosis of similar cell by polymorphonuclear leucocytes in the right fifth motor nucleus. Note general edema.

PLATE IV

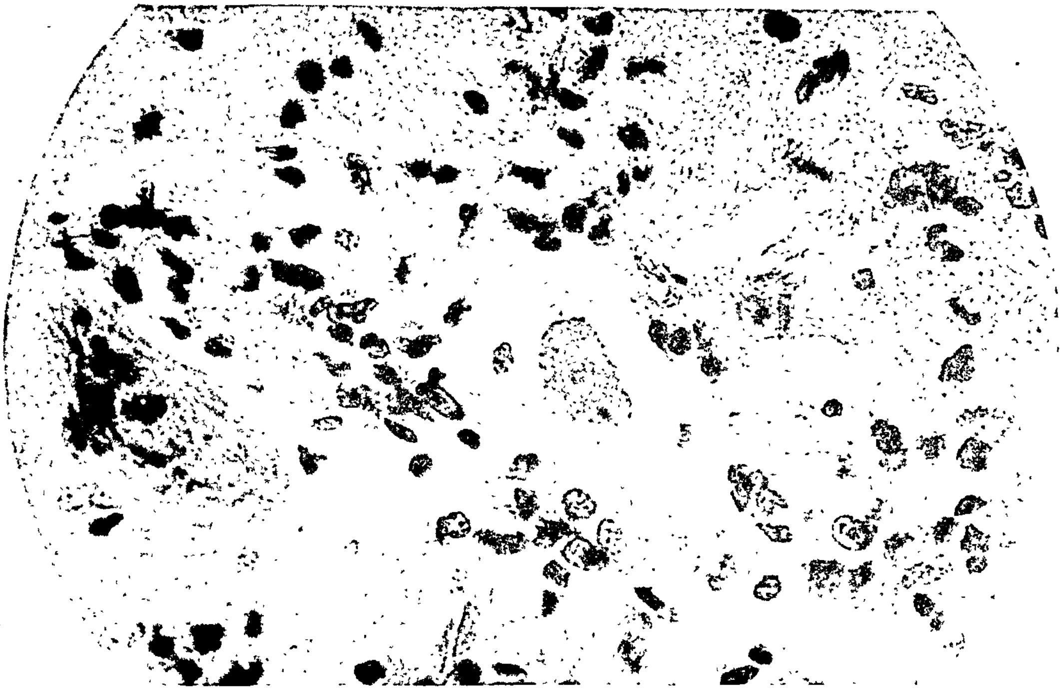
Fig. 5. R. 224. Drawing to show (*a*) intranuclear body in a cell of Schwann's sheath in the motor division of the fifth cranial nerve. The axis-cylinder (*b*) is faintly granular and vacuolated. The myelin sheath (*c*) appears intact.

Fig. 6. The same nerve showing mononuclear cell exudate about a nerve fiber, and an invasion of the myelin sheath by a phagocytic cell (*a*).



1

Goodpasture Portals of entry to the central nervous system



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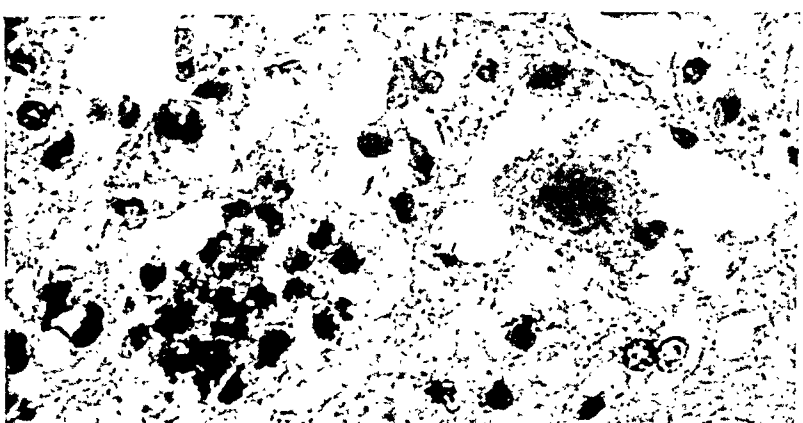

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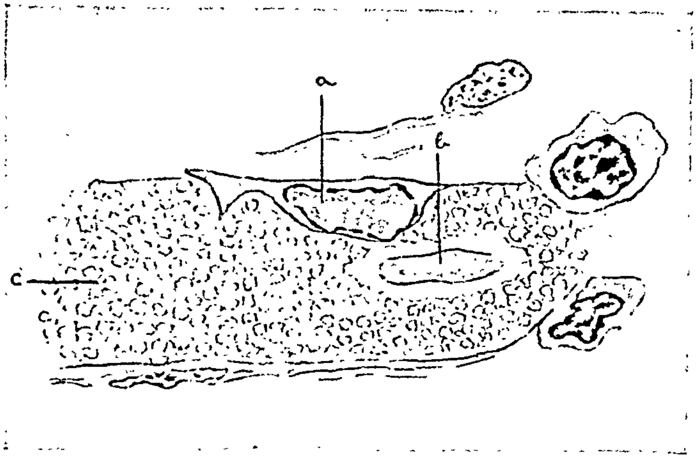

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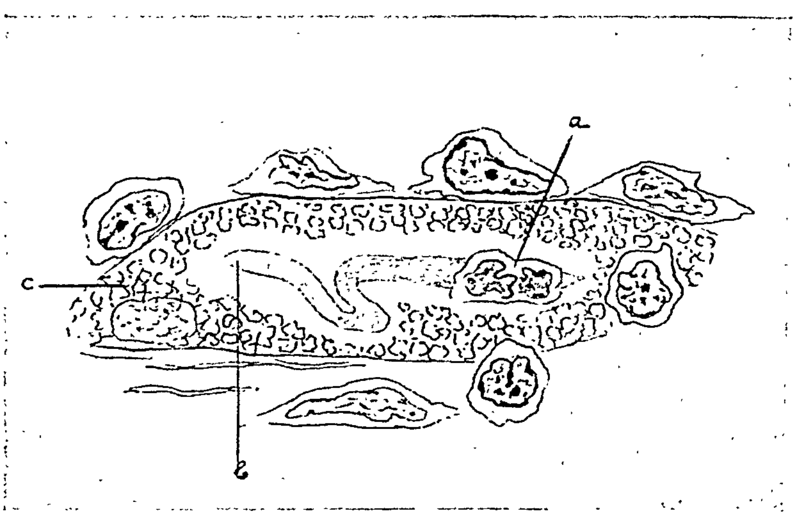

6

THE PATHWAYS OF INFECTION OF THE CENTRAL NERVOUS SYSTEM IN HERPETIC ENCEPHALITIS OF RABBITS CONTRACTED BY CONTACT; WITH A COMPARATIVE COMMENT ON MEDULLARY LESIONS IN A CASE OF HUMAN POLIOMYELITIS*

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Part I

The initial lesion in the central nervous system following a successful peripheral inoculation of rabbits with a strongly neurotropic strain (Strain M) of the virus of herpes simplex is so definitely related to the nerve supplying the inoculated area and its central termination,[1] that given suitable sections of a brain thus infected it is possible for one to determine not only by which nerve the virus gained entrance, but in certain instances in what peripheral distribution of a particular nerve the original infection was located.

By a study of the brain and spinal cord with particular reference to the entrance and central distribution of the cranial and spinal nerves it was thought possible to determine with certainty in this manner the mode of entrance of the virus in cases of contact infection, should rabbits acquire a herpetic encephalitis through exposure to an infected animal.

Normal rabbits proved to be very susceptible to contact infection with Strain M virus and a high per cent of those placed in the same cages with rabbits inoculated upon the cornea contracted in a few days a herpetic encephalitis. The first evidence of an infection of the brain was an elevation of the body temperature. A few animals were permitted to die of the disease and these for the most part showed much the same symptoms without evidence of a unilateral cerebral lesion.

When the body temperature first became elevated there were no other indications of disease. In the course of a day or two, however, there developed muscular weakness and tremors associated with a

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rather general clonic contraction of muscles. One animal turned its head to the right, in others the head was periodically drawn backward as in opisthotonos. These symptoms were rapidly followed by an apparent loss of equilibrium, weakness, convulsions and death. It is probable that a large per cent if not all of our animals that contracted herpetic encephalitis would have died of the disease in a short time though the mortality rate was not determined.

In investigating the paths of entrance of the virus into the central nervous system it was desirable to examine the brain very soon after the virus entered and before lesions other than those associated with the nerve through which the virus was admitted had developed to any great extent.

Experience with herpetic encephalitis following peripheral inoculation and infection had made it appear most certain that in an encephalitis induced through infection by contact the virus would enter the brain in the same manner, that is, along the course of one or more of the peripheral and probably the cranial nerves. With this in mind most of the animals in these experiments were killed from one to four days after the first elevation of body temperature, and the brains fixed immediately after exsanguination by injecting Helly's fluid through the carotid arteries. The brain and spinal cord were then removed in toto and the fixation continued for 24 hours in an ample amount of the same fluid. After washing, blocks including an entire cross section of the brain were cut from the olfactory and frontal lobes, the mid-cerebrum through the infundibulum, midbrain, pons, medulla and spinal cord at various levels. Microscopic sections soon revealed the two localities where herpetic lesions were to be expected, that is, at the entrance of the fifth cranial nerves in the pons and the ninth or glossopharyngeal nerves in the medulla. In succeeding experiments in addition to sections from the above-mentioned regions, serial sections were made including intradural portions of the fifth and ninth nerves and their entire sites of entrance and distribution in the brain.

In this way it has been possible to show that in herpetic encephalitis contracted through contact, bilateral herpetic lesions occur at the central terminations of one or both of these nerves, usually more marked on one side. In the case of the sensory division of the fifth nerve, lesions could be demonstrated within the nerve central to the plane where the sheaths of Schwann disappear and before its

entrance into the brain, as was likewise the case following inoculations of the cornea. However, in the spontaneous or contact infections, the lesion involved a different portion of the nerve, affecting fibers in the dorsal third, whereas following herpetic infection of the cornea the ventral portion of the nerve contained the lesion. This relation was also preserved in the descending crescentic tract, the lesion in contact infections occupying the dorsal horn as the bundle of fibers coursed through the medulla.

In two instances there was a lesion in or at the termination of that group of fibers which enters with the motor division of the fifth cranial nerve and arises from the unipolar ganglion cells situated at the lateral angles of the floor of the fourth ventricle, constituting the mesencephalic or accessory fifth motor nucleus. In one of these cases in which no lesion was present in the corresponding sensory division of the nerve there was no meningitis at its point of entrance. In all the other cases there was an acute meningitis at the entrance of the fifth or ninth nerves or both, as well as an exudate in the brain along their distribution. In those instances where the glossopharyngeal nerves were involved destructive lesions were found in the medulla restricted to the course of these fibers within the medulla and in the corresponding nuclei in the floor of the fourth ventricle.

In earlier stages of the lesions intranuclear herpetic bodies, especially within neuroglia cells, are very numerous, and there is an associated necrosis of cells with many pyknotic nuclear fragments, and a cellular exudate in which polymorphonuclear leucocytes are prominent. In the meninges at this stage there is usually in addition to cellular exudate, fibrin and red blood corpuscles. Fibrinous and hemorrhagic exudate may also be a part of the lesion within the brain.

In each instance it was possible clearly to associate the lesions found with the fifth or ninth cranial nerve. In one brain in which virus evidently entered through the sensory division of the fifth nerve, there was a question as to the origin of an acute herpetic lesion which was found at the site of entrance of one of the glossopharyngeal nerves. It was not possible to determine with certainty whether the latter was due to virus entering through the ninth nerve or to an extension of the infection outward along this nerve from a descending lesion in the sensory tract of the fifth nerve. The cause of confusion was due to the fact that in herpetic encephalitis con-

tracted through contact in which virus enters through the sensory division of the fifth cranial nerve the lesion in the nerve and in its descending tract occupies a dorsal position and passes through the medulla on a level with the ninth nerve as it enters, and only a thin layer of fibers of the Tractus spino-cerebellaris dorsalis[2] separates them from the periphery. However, when virus enters distinctly through the ninth nerve there is not only a peripheral lesion about the fibers of this nerve, but also a well-marked lesion in the corresponding nucleus at the floor of the fourth ventricle.

In later stages of the infection herpetic inclusions in the lesions at the site of entrance were rare or not found at all, the conspicuous feature being the large number of phagocytic mononuclear cells containing vacuoles in their cytoplasm indicating a removal of degenerated myelin sheaths. The initial lesions tend to heal in this way, though the virus extends rapidly to the base of the cerebrum, where its earlier stages of activity may be seen in the cells of the Area praesubicularis of the Lobus piriformis as in R. 221.

In no instance has a lesion in the olfactory lobes been found, and in this series of experiments the olfactory nerves may be entirely excluded from consideration as a portal of entry for the virus.

In most of the following experiments serial sections were made through the entrance of the fifth and ninth cranial nerves.

Experiment 1.

R. 182. Adult male.

4/17. Placed in cage with R. 260 (Herpes both eyes).
4/19. Temp. 103.3° F.
4/20. " 103°.
4/21. " 103°.
4/22. " 103°.
4/23. " 102.4°.
4/24. " 104.2°. On right side of tongue is an irregular ulcerated area about 5 mm. in diameter. Saliva inoculated upon scarified right cornea of R. 273.
4/25. Temp. 103.7° F.
4/26. " 103.4°.
4/27. " ?
4/28. " 100.5°. Very sick and weak. Unsteady equilibrium, difficulty in breathing. Died 1 P.M.
Autopsy negative. Brain shows a macroscopic hemorrhage in medulla on right side. Lesion on tongue healed.

R. 273. Half-grown rabbit.
4/25. Right cornea scarified and inoculated with saliva from R. 182.

4/28. Right eye greatly inflamed and closed with pus. Herpetic kerato-conjunctivitis.
4/30. Head turning to right, partial loss of equilibrium. Herpetic encephalitis.
5/4. Recovering.

Pons.

Serial sections through entrance of fifth cranial nerve. On the right side just median to the plane at which the sheaths of Schwann are lost and involving the dorsal one third of the root there is a large area of necrosis and cellular infiltration in the sensory bundle of the fifth nerve before it enters the brain. The predominant cell in the exudate is the large mononuclear phagocytic cell filled with small vacuoles. About the entering nerve there is a cellular exudate in the meninges consisting chiefly of mononuclears, though containing an appreciable number of polymorphonuclear leucocytes. The portion of the nerve containing sheaths of Schwann shows no change, and the motor root appears normal throughout. Blood vessels within the pons in the neighborhood of the entering nerve present a thin perivascular exudate of mononuclear cells.

On the left side there is a similar lesion occupying the corresponding portion of the sensory root on this side, though the extent of injury is considerably smaller. There is a local meningitis over the area of injury, and a moderate perivascular infiltration in the neighboring pons.

The pyramidal tracts on both sides, but more conspicuously on the right, are clear and the fibers appear vacuolated while neuroglia cells contain intranuclear herpetic bodies.

Medulla. On the right side occupying the dorsal portion of the descending bundle of sensory fibers of the fifth cranial nerve is a large area of necrosis with much hemorrhage and cellular infiltration, by both large mononuclear phagocytes and polymorphonuclear leucocytes. The lesion extends through to the lateral surface on this side and there is a cellular and fibrinous exudate in the meninges. The nucleus of the glossopharyngeal nerve is not involved. There is a small lesion having a similar situation in the fifth sensory tract on the left side and a slight perivascular exudate on this side.

There is a cellular exudate in the meninges over the ventral surface of the medulla and a perivascular infiltration about vessels dipping into the cerebral tissue.

In this case the virus entered the pons through the sensory portion of both fifth cranial nerves and extended downward bilaterally into the medulla in the sensory tracts. In contrast with the lesion of this tract following inoculation of the cornea the lesion is situated in the upper pole of the crescentic tract, and in the dorsal portion of the nerve root before it enters the brain.

The absence of involvement of the region of the glossopharyngeal nucleus excludes any probable entrance of virus through this nerve.

Experiment 2.

R. 221. Adult rabbit.

3/20. Placed in cage with R. 219 (Herpes right eye, inoculated 3/19).
3/21. Temp. 102.5° F.
3/24. Temp. 103°.
3/28. Temp. 105.1°. Nervous and shaky. Rapid respiration. Nose, mouth and eyes negative.

3/29. Temp. 104.5°. Holds head to right.
3/31. Temp. 105.3°. Holds head to right. Bloody discharge from rectum. Has lost considerable weight, salivated. Etherized. Autopsy showed acute intersusception of rectum. There are small hemorrhages superficially along the medulla on the right side.

Pons.

Serial sections through the pons at the entrance of the fifth cranial nerve show no lesion in the nerve on either side. There is slight cellular infiltration about the lateral surfaces of the floor of the fourth ventricle and slight perivascular infiltration. This area of inflammation on either side involves the accessory motor nucleus of the fifth nerve. No herpetic inclusions found.

There is an acute lesion along certain fibers of the right motor bundle characterized by edema and cellular infiltration with large phagocytic mononuclears containing small vacuoles. This occurs first about opposite the main motor nucleus and the cellular infiltration extends upward to a group of cells at the angle of the floor of the fourth ventricle. There is a lesser cellular infiltration similar in distribution on the left. The main motor nucleus with its multipolar ganglion cells is not involved on either side except for moderate perivascular infiltration on the right. No herpetic inclusions are observed, though the acuteness of the lesion and its general appearance indicate its herpetic origin.

Section of the medulla on a level with the entrance of the glossopharyngeal nerve shows at the point of entrance of this nerve on the right side a large acute destructive herpetic lesion with necrosis, hemorrhage, and cellular infiltration. Large mononuclear phagocytes containing vacuoles predominate, though there are many polymorphonuclear leucocytes. This lesion extends inward and dorsally across the fifth sensory tract for a considerable distance along the course of the ninth nerve, the fibers of which are destroyed. There is an interruption of the massive destruction separating the more peripheral lesion from the area of the ninth and tenth nuclei laterally at the floor of the fourth ventricle. This area shows a widespread but circumscribed destructive lesion. The entire area stains lightly and there is destruction within it of nerve fibers and neuroglia, most of the ganglion cells escaping. An abundant cellular exudate is present consisting for the most part of large mononuclear phagocytes. There is also a thick mantle of mononuclear cells about the blood vessels.

On the left side in the same sections there is a lesion of the same distribution though not so extensive and destructive.

Serial sections anterior to this region show a continuation of the area of necrosis and infiltration at the floor of the fourth ventricle but becoming smaller and more deeply situated. It comes to lie rather deeply in the medulla and more laterally, finally disappearing altogether further anteriorly. There is no evidence of a primary lesion in the descending tracts of the fifth nerve.

Posteriorly from the point of entrance of the ninth cranial nerve, the lesion at the floor of the fourth ventricle extends throughout the limits of the tenth nucleus, but diminishing in severity.

The sections indicate that the virus entered the brain in this case principally if not altogether along the glossopharyngeal nerves producing a lesion at their entrance and involving the nuclei of the ninth and tenth nerves on both sides.

In the previous sections the lesions are largely in a state of repair by phagocytosis.

Cerebrum through the infundibulum. Sections through the cerebrum on a

level with the infundibulum show an extensive acute herpetic encephalitis. There is a cellular exudate in the meninges about the infundibulum.

The acute herpetic lesion is sharply circumscribed on each side and more advanced on the right side. The cortical cells of the Area praesubicularis of the Lobus piriformis [2] contain intranuclear herpetic inclusions of various sizes and on the right many of these cells are undergoing necrosis and are calling out a mononuclear cell exudate. The area involved is sharply limited by the Fissura rhinica. On the left side the herpetic lesion has an identical distribution, but intranuclear bodies are less numerous and there is as yet very little necrosis. Sections through the olfactory lobes and tracts show no lesion.

Experiment 3.

R. 245. Normal large adult non-pregnant female.

4/3.	Placed in cage with R. 242 (Herpes right eye, inoculated 4/3).
4/4.	Temp. 102.8° F.
4/5.	" 102.8°.
4/7.	" 103.3°.
4/8.	" 103°.
4/9.	" 103.1°.
4/10.	" 104.9°. Eyes clear. No discharge from nose.
4/11.	" 103.7°.
4/12.	" 102.4°.
4/14.	" 105.8°. Eyes, nose and mouth clear. Very nervous, clonic contractions of muscles, sick. Etherized. Pharynx, naso-pharynx, larynx, buccal mucosa, trachea, esophagus and stomach are smooth and pale, showing no evidence of inflammation. Autopsy negative.

Olfactory Lobes. Sections through the olfactory lobes and tracts show no lesion.

Cerebrum. Sections through the cerebrum passing through the infundibulum show no lesion.

Pons. Sections through the pons at the entrance of the fifth nerves show a large destructive herpetic lesion occupying the position of the dorsal third of the sensory division on the right side central to the plane where Schwann's sheath is lost. There is destruction of fibers and myelin sheaths, and a cellular infiltration, with mononuclear phagocytes predominating.

Medulla. Serial sections through the medulla at the entrance of the ninth and tenth cranial nerves show a small herpetic lesion in each descending tract of the fifth cranial nerve. There is also a slight cellular exudate and perivascular infiltration in the nuclei of the ninth and tenth nerves at the floor of the fourth ventricle, but no lesion in relation to these nerves as they enter the medulla.

Cervical Cord shows no lesion.

In this case the virus entered the brain mainly through the sensory division of the right fifth cranial nerve, and probably to a less extent through the left. Possibly virus entered also to a certain extent through the ninth nerve.

Experiment 4.

R. 246. Large adult non-pregnant female.

4/3.	Placed in cage with R. 244 (Herpes right eye, inoculated 4/3).
4/4.	Temp. 102.8° F.

4/5. Temp. 102.8°.
4/7. " 103.1°.
4/8. " 102.8°.
4/9. " 103.1°.
4/10. " 102.7°.
4/11. " 103°.
4/12. " 103.8°.
4/14. " 105.1°. Eyes, ears and nose clean. No noticeable symptoms.
3 P.M. Temp. 106.4°, respirations rapid. No evidence of muscular involvement. Etherized. Autopsy negative.

Sections through the olfactory and frontal lobes, cerebrum at the infundibulum, and cervical spinal cord show no lesions.

Pons at entrance of fifth nerve. On the left side occupying the dorsal third of the sensory division of the fifth nerve just central to the plane where the sheaths of Schwann are lost, there is a large acute herpetic lesion showing destruction of fibers and myelin sheaths, and cellular infiltration principally with large mononuclear phagocytes filled with vacuoles. There is also a moderate mononuclear cell exudate in the meninges over this area. There is no lesion of the motor root. From the point of entrance into the pons of the fifth nerve upward and inward to the angle of the floor of the fourth ventricle there is a mononuclear perivascular infiltration; and involving the region of the accessory motor nucleus, the Nucleus ventralis Bracchii conjunctivi and the Bracchium conjunctivum cerebelli there is a diffuse cellular infiltration and focal groups of large mononuclear phagocytes.

On the left side there is no lesion in the sensory division of the fifth nerve. In the motor nerve, however, in the bundle of fibers which passes beyond the main motor nucleus to end in the accessory motor nucleus at the angle of the floor of the fourth ventricle, there is an acute herpetic lesion at a point just dorsal to the main motor nucleus. Here there are numerous neuroglia cells containing intranuclear bodies and an infiltration with mononuclear phagocytes. There is also a perivascular infiltration in the neighborhood of the lesion.

Sections through the pons at the entrance of the eighth nerve show only a lesion in the dorsal horn of the crescentic sensory tract of the fifth nerve in the left side, a descending continuation of the lesion in the nerve at its entrance. There is no similar lesion on the right side.

Medulla. Serial sections through the medulla show a moderate cellular infiltration of the median portion on each side and here and there a focus of leucocytes. At the entrance of the glossopharyngeal nerve in the left side there is a destructive herpetic lesion extending inward along the course of entering fibers through the sensory tract of the fifth nerve. About the entrance of the nerve there is a cellular exudate in the meninges.

With a narrow interruption the lesion involves the ninth and tenth nuclei at the base of the fourth ventricle. Here the lesion is largely interstitial consisting of a destruction of fibers, myelin sheaths and neuroglia, and a cellular exudate and perivascular infiltration.

A similar though less extensive lesion involves the entrance of the ninth nerve on the right side, and the corresponding nucleus on this side.

In this case virus entered the pons through the sensory division of the left fifth cranial nerve, and probably through those fibers of the motor divisions of this nerve on each side which terminate in its mesencephalic or accessory nuclei.

In the medulla, virus entered through the glossopharyngeal nerve on each side producing the greater injury on the left side.

Experiment 5.

R. 247. Normal large adult male.

4/3. Placed in cage with R. 243 (Herpes right eye, inoculated 4/3).
4/4. Temp. 102.9° F.
4/5. " 102.8°.
4/7. " 103.2°.
4/8. " 103.3°.
4/9. " 103°.
4/10. " 103°.
4/11. " 102.4°.
4/12. " 102.6°.
4/14. " 102.4°.
4/15. " 102.8°.
4/19. " 102.4°. Remained normal.

Experiment 6.

R. 268. Pregnant white adult female.

4/18. Placed in cage with R. 267 (Herpes right eye, inoculated 4/18).
4/19. Temp. 103° F.
4/20. " 103.1°.
4/21. " 103°.
4/22. " 103°.
4/23. " 103.1°.
4/24. " 103.4°.
4/25. " 103.1°.
4/26. " 102.8°.
4/29. " 103°. Placed in cage with R. 283 (Herpes right eye, inoculated 4/29).
4/30. Temp. 102.8°.
5/1. " 102.8°.
5/2. " 102.8°.
5/3. " 102.5°.
5/14. " 104.8°. Aborted young almost term. Holds head backward, weak and shaky. Emaciated.
5/16. Dead, encephalitis. Autopsy grossly negative.

Experiment 7.

R. 270. Young adult rabbit.

4/20. Placed in cage with R. 269 (Herpes right eye, inoculated 4/20).
4/22. Temp. 102.8° F.
4/23. " 102.8°.
4/24. " 103°.
4/25. " 102.8°.
4/26. " 103.2°.
4/28. " 106°. Etherized. Eyes, ears, nose, and mouth clean. Autopsy negative.

Pons at entrance of fifth cranial nerves. On the right side involving the dorsal half of the sensory division of the fifth nerve there is a large diffuse and very acute herpetic lesion. Innumerable neuroglia cells contain herpetic inclusion-bodies and there is a moderate polymorphonuclear and mononuclear leucocytic exudate and many small hemorrhages. About the entrance of the nerve there is a mononuclear cell exudate in the meninges. No lesion is observed in the fifth nerve on the left, and there is no other lesion in the brain at this level.

Sections of the pons at the entrance of the eighth cranial nerves show an acute herpetic lesion similar to that found in the above sections, occupying the dorsal horn of the right descending tract of the sensory division of the fifth cranial nerve. At this level there is a similar lesion in the corresponding right descending sensory tract. Both these lesions are very acute, presenting numerous cells with herpetic inclusions in their nuclei, and a cellular exudate in which polymorphonuclear leucocytes are prominent.

Medulla. Sections through the medulla including the entrance of the ninth cranial nerve show on both sides an acute herpetic lesion at the entrance of these nerves, and to a lesser degree in the corresponding nuclei at the floor of the fourth ventricle. In the meninges at the entrances there is an acute exudate of mononuclear and polymorphonuclear leucocytes. Within the medullary tissue along the distribution of the fibers of these nerves, but most marked at the periphery, there is an acute herpetic lesion. Innumerable neuroglia cell nuclei contain herpetic inclusions and similar inclusions are found in neuroglia cells in the ninth nucleus on the right side. In both ninth nuclei there is a moderate diffuse cellular exudate. No lesions are observed elsewhere.

In this case herpetic virus entered the brain through the sensory division of the right fifth cranial nerve, and through both glossopharyngeal nerves. The lesions everywhere are very acute.

Experiment 8.

R. 272. Adult rabbit.

4/25.	Placed in cage with R. 271 (Herpes right eye, inoculated 4/24).
4/26.	Temp. 103° F.
4/29.	" 104°.
4/30.	" 104.4°.
5/1.	" 104.3°. Etherized. Autopsy negative.

Microscopic Notes. Sections through frontal lobes, cerebrum at infundibulum, pons and medulla show no evidence of herpetic infection of the brain.

This animal did not have herpetic encephalitis histologically. The rapid onset of elevated temperature and the fact that the fever remained low for three days suggests that some other cause than an herpetic infection was responsible for it.

Experiment 9.

R. 278. Adult pregnant female.

4/26.	Placed in cage with R. 274 (Herpes both eyes, inoculated 4/25).
4/29.	Temp. 102.5° F.
4/30.	" 102.4°.
5/1.	" 103.9°.
5/2.	" 102.6°.

5/3. Temp. 102.8°.
5/14. " 102.6°.
Tested for immunity.
6/4. Right eye inoculated with pus from eye of R. 308.
6/9. Temp. 107.2°. Head drawn strongly to the right.
6/10. Lying in cage on right side. Complete loss of equilibrium.
6/13. Dead. Encephalitis.

Discussion. In the above nine experiments in each instance a single normal adult rabbit was placed in the same cage with a rabbit that had been inoculated on the right cornea, within 24 hours previously, with the virus of herpes simplex and had developed as a consequence a severe herpetic kerato-conjunctivitis with a copious discharge of infectious fluid exudate and pus. In an additional case a mother contracted a fatal herpetic encephalitis from contact with her young similarly inoculated on the cornea. Microscopic sections of the brain proved the herpetic nature of her encephalitis but no attempt was made to study the relation of the lesions to nerves.

Thus of ten experiments in which normal rabbits were placed in contact with rabbits infected in the eye, seven contracted a herpetic encephalitis, and three of these died of the disease, four were killed a short time after the first appearance of symptoms. One rabbit which showed a slight elevation of temperature on the fourth day after exposure was killed two days later and the brain revealed no evidence of herpetic infection. Two rabbits after exposure for several days showed no symptoms of herpetic infection. Six weeks later one of these was inoculated upon the eye with herpetic virus and died nine days later with herpetic encephalitis, thus showing no evidence of immunity to the virus.

Each of the seven animals that contracted herpetic encephalitis showed an elevation of body temperature within a few days after exposure, the average being eleven days. This average is high because of one animal whose first elevation of temperature occurred on the twenty-sixth day, in the others the average was eight days. The three animals that died of the disease lived from two to five days after the first recorded elevation of body temperature.

Five rabbits were killed from one to four days after the onset of fever, and serial sections were made through the pons and medulla, in addition to routine cross sections through other portions of the central nervous system. In these cases it was clearly shown that the virus entered the brain twice through the sensory division of the

fifth cranial nerves, once through the ninth cranial nerves, and twice through both these nerves. In two cases in addition to other lesions there was a herpetic inflammation involving the accessory motor nuclei at the lateral angles of the floor of the fourth ventricles. Virus apparently entered by way of the motor nerve through the fibers which have their origin in these nuclei. The exact peripheral distribution of these fibers does not seem to be accurately established, though according to Terterjanz [3] they supply the tensor palati muscle. The possibility of sensory fibers entering the brain with the motor bundle, along which virus might pass, is to be considered, particularly inasmuch as one usually finds a few scattered ganglion cells within the proximal extra-cerebral portion of this nerve.

In each of the encephalitic brains the lesions were bilateral, either involving both the fifth and ninth nerves on each side, or one on one side and one on the other. The short duration of life in the three animals dying from the disease is to be attributed to the extensive involvement of the medulla.

There can be no reasonable doubt in the above cases, in which the central distribution of the cranial nerves was studied, that the herpetic virus entered the brain along either or both the fifth and ninth cranial nerves. Consequently the peripheral source of the virus must have been in the region of the peripheral distribution of these nerves, that is to say, in the case of the sensory division of the fifth nerve most probably in the mucous membrane of the mouth or nose, and in the case of the ninth nerve in the mucous membrane of the posterior portion of the tongue or of the pharynx.

In one case it was almost certain that the infection originated, in part at least, in a lesion on the right lateral surface of the anterior third of the tongue. In R. 182 five days before death a small ulcer was found at this site, and saliva removed at the same time and inoculated upon the cornea of R. 273 induced the usual herpetic keratitis and symptoms of encephalitis from which the rabbit recovered.

In the other animals no lesion of the oral or nasal mucosa was discovered at autopsy, and at the time of death the lesion on the tongue of R. 182 had healed.

Previous experiments of inoculating herpetic virus into various organs and tissues have forced the conclusion that a peripheral take is a necessary preliminary to an invasion of the central nervous system by the virus. It is assumed likewise in herpetic encephalitis

contracted by contact that there is always an initial herpetic lesion in the mucosa of the mouth, nose, or throat from which virus extends along neural fibers innervating this area, into the brain, though this point has not been thoroughly established. It must be borne in mind that an inconspicuous lesion of the mucous membrane may heal readily, so that it may be overlooked or absent at autopsy, yet virus should be demonstrable in the saliva or naso-pharyngeal secretions before encephalitis becomes manifest. This phase of the problem has not yet been investigated beyond the one experiment above mentioned.

As to the manner of transmission of herpetic virus from one animal to another in contact in these experiments, there are the possibilities that it was acquired through contamination of food with the infected secretions, or from licking the infected eye. Small abrasions of the mucous membrane of the mouth, nose or throat would readily lead to a local herpetic infection of the epithelium. This has been demonstrated by lightly scarifying the mucosa inside the cheek and applying to it infected pus from an experimental herpetic keratitis. Sections of the inoculated mucosa removed 24 hours later showed an area of ulceration about which the epithelial cells contained in great numbers the typical intranuclear inclusions of Lipschütz.

In view of these experiments, herpetic encephalitis may be considered a contagious disease for rabbits which are intimately exposed to the virus. There is probably in every case a primary infection of epithelial cells in the mucosa of the mouth, nose or throat. Depending on the situation of such a primary infection the virus propagates itself along the axis-cylinders of sensory nerves supplying the infected tissue until it reaches the central nervous system, usually by way of the fifth and ninth cranial nerves.

PART II

MEDULLARY LESIONS IN A CASE OF HUMAN POLIO-ENCEPHALO-MYELITIS

The possibility of the passage of the virus of poliomyelitis under experimental conditions from the periphery along nerve fibers to the central nervous system has been demonstrated by the investigations of Flexner and Lewis,[4] and of Levaditi and Landsteiner.[5] If one introduces the virus into a peripheral nerve the animal becomes para-

lyzed first in the muscles supplied by this nerve. Following inoculation of the virus into the naso-pharyngeal mucosa the resulting disease manifests itself first by paralysis of muscles of the neck and upper extremities, or inoculated into the mucosa of the intestine the paralysis is first evident in the lower extremities.

These facts point directly to an invasion of the central nervous system by way of nerve fibers under the conditions of the experiment, yet it has not been determined that a similar portal of entry into the central nervous system is involved under the natural conditions of infection in the human being.

The demonstration anatomically of the relation of early lesions in the central nervous system in herpetic encephalitis acquired by contact to the central terminations of the fifth and ninth cranial nerves suggests that a study of suitable cases, especially those succumbing to the disease in its earlier stages, may establish a similar relation of the initial lesions to certain peripheral nerves as portals of entry for the virus of poliomyelitis in man.

An opportunity was afforded recently to study histologically the brain of a sporadic case of polio-encephalo-myelitis in which lesions are present in the medulla that are strikingly analogous to those present in a similar situation in rabbits which have acquired herpetic encephalitis through an invasion of the brain by way of the glossopharyngeal nerve. Sufficient material was not obtained from this case to make a complete study of the central terminations of peripheral nerves, as the diagnosis at the time of autopsy was not established, and the poliomyelitic nature of the disease was demonstrated only by microscopic sections.

The lesions demonstrated, however, are sufficiently suggestive of a relation to the glossopharyngeal and vagus nerves in the medulla to warrant description. Occasion is also afforded to emphasize the desirability of studying the brains of similar cases from the standpoint of determining the relation of the earliest central lesions to the entrance and proximal distribution of peripheral nerves which may serve as portals of entry for the virus of poliomyelitis, especially the fifth, ninth and tenth cranial nerves.

Patient, J. C., a white boy aged 17 years, entered the hospital complaining of having had "sore throat," and at present an inability to swallow. He had had tonsillitis ten years ago and following this his tonsils were removed.

A little over one week ago he had a slight "sore throat" accompanied by

headache, and he thought he had "grippe." On examination at this time his physician found no demonstrable lesion in the throat or pharynx. His appetite was poor and he had been unable to swallow for seven days. Bowels had been regular. There was a slight cough.

On physical examination the ears and nose were negative. The eyes reacted normally. The throat appeared congested and swollen beneath the soft palate on the right. The breath sounds over the chest were very harsh, especially anteriorly over both sides above. There were many dry rales on expiration. Vocal fremitus and tactile fremitus were increased. Heart sounds regular. Abdomen and extremities were negative.

Ten hours later (10 P.M. on the day of admission) the patient was irrational and restless. Frothy mucus was present in the mouth, and he had some cough and expectoration. On attempting to swallow water it ran from the mouth. There was no stertor or cyanosis. There was a generalized bronchitis, much more marked on the left, and there was dullness at the right base posteriorly. Heart was regular; the abdomen retracted; and the tissues much dehydrated. No muscular paralyses were observed in the throat or elsewhere.

The temperature on admission was 98.6° F., rapidly rising to 104° F. a few hours later.

The patient rapidly became worse, at one time was described as kicking and scratching wildly, and died at 8 A.M., 20 hours after entering the hospital.

While in the hospital the temperature arose from 98.6° F. to 106° F., pulse from 112 to 160, and respirations from 22 to 46 per minute.

Cultures from the throat on admission to the hospital and after death were negative for Bacillus diphtheriae.

Necropsy was performed two hours after death. There was found an extensive acute bronchopneumonia, bilateral in distribution, cultures from which yielded Staph. aureus and Pneumococcus Type IV. Heart's blood remained sterile.

In addition to the bronchopneumonia a small hemorrhagic area was found in the gross specimen of the medulla. A brief description of the brain is as follows: Wt. 1400 gms. The blood vessels of the brain are deeply congested and there appears to be a cloudiness of the fluid about the vessels. On section through the medulla there is a reddish-purple area 2 mm. in diameter present in the central part of the right side. This appears to be an area of hemorrhage and it extends for about 0.5 cm. parallel to the long axis of the medulla.

Portions of the medulla including a part of the area in which the hemorrhage occurred were ground and inoculated into the brains of two rabbits. These animals remained normal.

Microscopic sections of the brain show widely spread lesions which are regarded as those of polio-encephalo-myelitis.

Lesions of this nature are recognizable from the basal ganglia to the cervical cord, but are most conspicuous and destructive in the medulla on a level with the entrance of fibers of the ninth and tenth nerves and their nuclei at the floor of the fourth ventricle.

In the basal ganglia only an occasional focus of large mononuclear phagocytic cells is recognizable, and in the neighborhood of such foci there is a moderate lymphocytic infiltration about small blood vessels.

In sections of the cervical portion of the spinal cord there is a quite marked destructive lesion in the ventral horns, characterized by a general mononuclear

phagocytic cell infiltration, perivascular accumulation of lymphocytes, and destruction with phagocytosis of an occasional necrotic motor ganglion cell.

In the medulla, however, corresponding to the position of the hemorrhage observed in the gross specimen, there is a very acute and profoundly destructive inflammatory lesion involving both sides but more marked on the right.

On this side the most conspicuous change is a large area of necrosis and hemorrhage which has become infiltrated by mononuclear phagocytes with vacuolated cytoplasm, no doubt from the removal of disintegrating myelin. The lesion is thus in the reparative stage, and resembles very strikingly lesions occurring in a similar situation in later stages of contact herpetic encephalitis in rabbits. The lesion is situated a little dorsal to the center of this side of the pons above

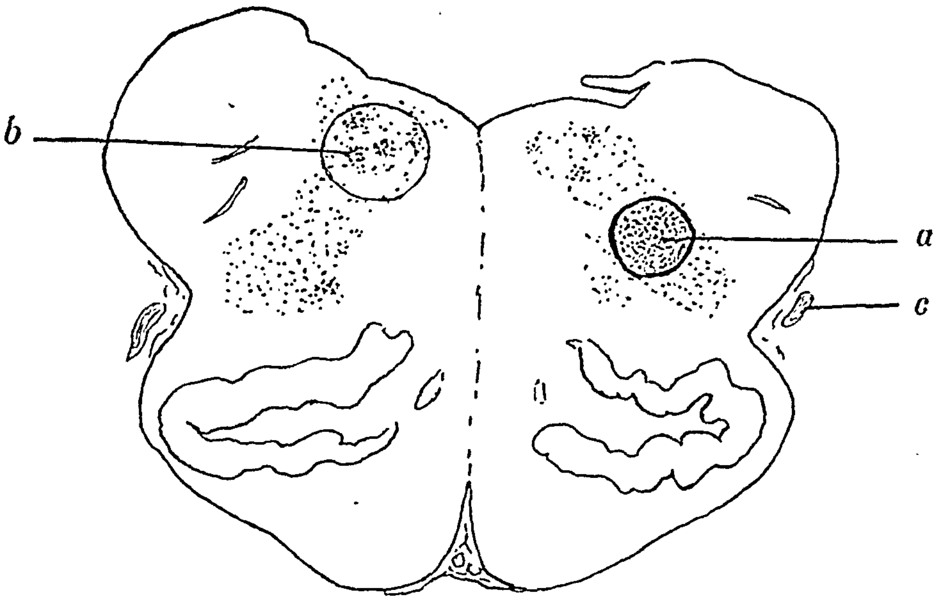

FIG. 1. Schematic drawing of a section through the medulla of a case of polio-encephalo-myelitis. (*a*) is an area of necrosis and hemorrhage. (*b*) is an area of necrosis and cellular infiltration. The dotted areas on each side outline the zones of inflammation corresponding to the distribution of the glossopharyngeal and vagus nerves and their nuclei at the floor of the fourth ventricle. (*c*) is a portion of the glossopharyngeal nerve.

the olive, and includes in its boundaries the approximate position of the Nucleus ambiguus. In addition to this lesion there is an extensive cellular exudate and perivascular infiltration occupying the area of the nuclei of the ninth and tenth nerves on both sides but more extensive on the left side. On this side in addition to a diffuse cellular exudate there is destruction and phagocytosis of ganglion cells within the nucleus.

At this level of the medulla the glossopharyngeal nerves enter in the postolivary sulcus, and although it has been impossible because of insufficient material to trace these nerves completely, there is evident on each side a distribution of cellular exudate and perivascular infiltration from the periphery about the sulci inward and upward to the corresponding ganglia at the floor of the fourth ventricle. On the right side the large area of hemorrhage and necrosis is included in this inflammatory zone. The pathological changes are such as to give a convincing impression that the virus entered the brain through the ninth

or tenth cranial nerves or both, produced its most extensive injury along their central distribution, and spread from this point up and down the cerebrospinal axis. (Text figure 1.)

The extent of the medullary lesions was such as to bring about death of the individual early in the disease. The difficulty in swallowing probably was a result of these lesions and contributed no doubt to the rapid onset of bronchopneumonia which terminated fatally.

In a case of poliomyelitis described by Landsteiner, Levaditi, and Pastia [6] there was evidence that the nasopharynx and tonsils were the portals of entry for the virus. In their case there was an acute inflammation in this region and after death poliomyelitic virus was demonstrated in the tonsillar tissue and in the nasopharyngeal mucous membrane by inoculations into monkeys, but none was demonstrable in cervical lymph nodes or salivary glands.

While in the majority of cases of poliomyelitis lesions in the medulla are undoubtedly not so evident or extensive as in the one we have described, it is nevertheless possible that the regular route of invasion of the central nervous system is by way of the nerves supplying the mucous membranes of the mouth, nose and pharynx, as in contact infection with herpes. A small local lesion at the site of entry of these nerves into the brain could undoubtedly serve as a center from which the virus would spread rapidly, attacking the nervous structures which are most susceptible to its effects. An analogy is to be found in herpetic encephalitis where an initial lesion at the site of entry of a particular nerve may be small and in a state of repair, while a diffuse acute infection of the basal cerebral cortex leads to death of the animal.

Summary

1. Normal rabbits readily contract herpetic encephalitis when placed in the same cages with rabbits inoculated on the cornea with the virus of herpes simplex (strain M).

2. There is probably in every case an initial herpetic infection in the mucous membrane of the mouth, nose or throat.

3. The virus reaches the central nervous system through the sensory division of the fifth and the ninth cranial nerves.

4. Herpetic lesions have been demonstrated microscopically in association with the central termination of these nerves in the pons and medulla.

5. In no instance in these experiments has a lesion been observed in the olfactory lobes, or in association with any other cranial or spinal nerve.

6. A case of polio-encephalo-myelitis in a boy is described in which medullary lesions were found which appear to be directly related to the central distribution of the ninth and tenth cranial nerves. It is suggested that the virus of poliomyelitis in human infections may enter the brain through peripheral nerves.

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CERTAIN FACTORS DETERMINING THE INCIDENCE AND SEVERITY OF HERPETIC ENCEPHALITIS IN RABBITS *

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In the course of passing the virus of herpes simplex from rabbit to rabbit during the past eighteen months several observations of interest have been made relating to the natural resistance of these animals to herpetic encephalitis. In this paper it is proposed to record such as have been controlled by appropriate experimental tests, especially the facts thus established that have a bearing on the relation of the strain of virus, the size of the peripheral herpetic infection experimentally induced, and the age of the animal, to the incidence and severity of herpetic encephalitis.

It was early recognized that not all rabbits infected with the virus of herpes, even with a highly neurotropic strain, acquire as a result a herpetic encephalitis, and that an encephalitis once acquired is not invariably fatal. It became evident that the incidence and severity of herpetic encephalitis were controlled by two groups of factors, that is (1) those depending upon the virus used, and (2) those depending upon the animal sustaining the infection. It has been possible to analyze certain of these factors in each group, which for convenience may be described under the following headings.

I. FACTORS DEPENDING UPON THE VIRUS

(1) *Virulence of the strain of virus.* With this particular virus virulence so far as its propensity for invading the central nervous system and inducing encephalitis is concerned may be regarded as synonymous with what has been termed its "neurotropism," a characteristic which is poorly understood, but which in final analysis may be found not to be a specific affinity of the virus for nervous tissue, but a property dependent upon conditions which render cells of the central nervous system more readily invaded by certain strains than by others. It has been shown for instance that a strain of the

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virus of herpes simplex which was never observed to cause an encephalitis following a peripheral infection, was nevertheless able to proliferate locally following primary inoculation not only in nervous tissue but in derivatives of all three embryonic cellular layers, ectoderm, endoderm, and mesoderm;[1] and as will be shown below, an infection upon the cornea with a highly "neurotropic" strain does not necessarily lead to an encephalitis, provided the site of inoculation is a sufficiently small one. The virus of herpes simplex thus appears to be able to infect the cells of any tissue of the body if a favorable initial injury is induced at the time of inoculation.

Yet different strains of this virus exhibit a notable variation in their capacity to induce herpetic encephalitis from a peripheral infection. This has been demonstrated particularly well by inoculations upon the scarified cornea, using two strains of virus. The one, virus F, regularly produced a purulent kerato-conjunctivitis apparently of an equal intensity to that resulting from an infection with the other, virus M, and yet never leading to an encephalitis. Virus M, on the contrary, has consistently caused an infection of the central nervous system when a uniform technique was followed in inoculating the cornea. Virus F, however, when introduced directly into the brain usually caused a fatal infection, yet not invariably so.

The incidence and severity of an encephalitis are thus determined by some quality of the virus enabling it to penetrate nerves and propagate itself along them to the brain or spinal cord, a quality which varies widely in different strains.

(2) *Size of the initial peripheral infection.* When a uniform technique is followed for inoculating a rabbit's cornea for passage of the virus, consisting in anesthetizing the animal with ether, evulsing the bulbus and making from five to six fairly deep parallel incisions entirely across the cornea and following these with similar incisions at right angles, then inoculating with pus from an eye infected in the same way 48 hours previously, by rubbing the inoculum into the incisions, an encephalitis invariably follows the kerato-conjunctivitis thus induced in adult rabbits, and is fatal in seven to nine days except in certain instances to be mentioned below.

Having established such a procedure, the results of which could be very accurately predicted, it was possible to vary both the conditions of the virus inoculated, using the same neurotropic strain, as well as the size of the area scarified, and in this way to estimate

the relative importance of each. It had been observed, especially when rabbits not full grown were used for transfer, that the inoculation with pus from an eye infected more than 48 hours previously resulted sometimes in an encephalitis which was not fatal, and for each 24 hours succeeding the first 48 hours the chances of inducing a fatal encephalitis became rapidly less, so that usually it was difficult to induce even an herpetic keratitis, using for the inoculum the pus from an eye infected five days previously.

This rapid reduction in the virulence of pus from an infected eye is due, we believe, to a diminution in the quantity of active virus and is directly proportional to the presence of cells, in the inflamed eye, which contain herpetic nuclear inclusions. These inclusions have been regarded by Lipschütz and by Goodpasture and Teague as representing the presence and growth of the virus within the nucleus.

At the end of the first 24 hours after inoculation the virulence of the secretion from the eye is apparently at its maximum, a time coincident with the full development of the clear herpetic vesicles along the lines of scarification. There is little or no exudate from the eye at this time, and it is, therefore, more convenient for practical purposes to use virus contained in pus 24 hours later or 48 hours after inoculation, as this is easily manipulated, and may be preserved in a convenient form in dilute glycerol.

It has been observed that an inoculation of the cornea with pus removed from an eye 72 hours or later after primary infection usually induces a milder keratitis as well as a less severe attack of herpetic encephalitis, and this is assumed to be due to a diminution in the amount of virus present in the exudate with a resulting infection only at certain points along the lines of scarification and a tendency for the infection to remain localized in these particular areas of corneal epithelium. The following experiments were performed to test this point.

Experiment 1. Two adult non-pregnant rabbits were inoculated on the right cornea in a scarified area 1 mm. square, near the corneal margin in the upper quadrant, with 24 hour virus from the eye of R. 297. A third rabbit was inoculated with the same virus after scarifying the cornea in the usual way for passage, as a control. The control died on the ninth day after the inoculation, with herpetic encephalitis. One of the two other rabbits died of encephalitis on the fourteenth day, the remaining one recovered after a severe attack of encephalitis.

This experiment shows that the virus from an eye inoculated 24 hours previously may produce a fatal encephalitis following even a very small initial inoculation on the cornea, but not constantly.

Experiment 2. Two adult male rabbits were inoculated on the right cornea in the center by making a fairly deep incision 2 mm. in length and patting into the incision a drop of pus removed from an eye inoculated 48 hours previously. As a control a third rabbit was inoculated with the same pus on the right cornea after scarifying in the routine way for passage.

The control rabbit died of herpetic encephalitis nine days later.

Neither of the other two rabbits showed an elevation of body temperature, turning of the head or any evidence of encephalitis. Typical vesicles, however, developed at the site of inoculation within 24 hours and two days later a secondary crop of vesicles appeared in the upper quadrant of the eye of one rabbit and in the posterior quadrant of the eye in the other rabbit. There was a mild conjunctivitis with congestion and lacrimation, but little pus. Ten days after inoculation the eyes were completely healed and 30 days later these animals were found to be immune to corneal infection with the same virus.

This experiment shows that notwithstanding a local herpetic lesion on the cornea, virus M does not necessarily invade the central nervous system, provided the initial site of scarification is small enough and 48 hour virus is used. The herpetic infection exhibits little tendency to spread locally, remaining quite sharply confined to the area inoculated. Isolated vesicles, however, do appear within areas corresponding apparently to the drainage by the corneal lymph spaces.

A recognition of this limitation of the local infection is of importance in understanding the nature of a local herpetic lesion, and of the manner in which the virus extends to the central nervous system. The infection tends to remain localized immediately to the cells about the point injured. The virus proliferates just as actively and as abundantly at the site of a small incision as it does proportionally in more extensive scarifications, but evidently the initial injury and infection must involve a certain minimum area or number of nerve fibers to result in a proliferation of the virus within axis-cylinders and along them to the brain to result in an encephalitis. And even though virus reaches the brain a minimum amount or a minimum extent of the initial intracerebral lesion is necessary to cause a fatal infection, for, as it is the transmission of the virus along the fifth cranial nerve from the cornea to the pons which initiates an herpetic lesion in the central distribution of this nerve, so in a fatal infection following corneal inoculation there is a secondary distribution of the virus from this initial lesion in the pons to the cerebrum

which brings about a fatal termination. Presumably if the lesion in the pons is small enough, a fatal encephalitis will not follow.

It is to be noted that in the two sets of experiments above recorded different areas of the cornea were scarified, in the first set an area near the conjunctival margin, in the second an area of approximately the same size in the center of the cornea. About the periphery of the cornea there is a rich plexus of nerves, the plexus annularis, from which fibers pass inwardly losing their medullary sheaths a short distance from the corneal margin. An inoculation near the periphery therefore would probably expose to the virus a greater number of nerve filaments than a similar inoculation nearer the center. This anatomical arrangement of the nerve fibers may explain the fact that encephalitis resulted in both animals of the first set of experiments and in neither of the second. It may thus be that of much greater importance than the size of the peripheral inoculation from the standpoint of the incidence of encephalitis is the number of nerve fibers involved and their degree of injury.

On this principle it is possible to immunize rabbits against a highly neurotropic strain of virus by vaccinating them in a small area relatively free of nerves with the same virulent strain, and without a likelihood of inducing thereby an encephalitis.

It may be stated as a result of these experiments that with a particular strain of herpetic virus the incidence and severity of encephalitis following corneal inoculation is within limits directly proportional to the size and extent of the initial corneal infection. This probably means that whether or not an encephalitis results depends on the extent of susceptible nervous tissue actually exposed to the peripheral infection, and on the fact that the virus in a local peripheral focus of infection will not spread far to infect neighboring cells and nervous tissue from which it might proceed to the brain.

II. Factors Depending upon the Individual Rabbit Infected

In the great majority of cases in our experiments herpetic virus has been passed for preservation in an active state from one adult rabbit to another by means of corneal inoculations. Under these circumstances the occurrence of a fatal encephalitis has quite uniformly followed where virus in 24 or 48 hour fresh exudate from an

eye was used for the inoculum after extensive scarification of the cornea. A particular strain of rabbits to be described below presented an exception. Occasionally, however, young animals of various ages have served for transfer, and with these the severity of the encephalitis which always occurred varied very greatly, and several groups of experiments have definitely proved that young rabbits of susceptible parentage are very resistant to herpetic encephalitis and rarely die of the disease, though the extent and severity of the corneal infection is proportionately as great as in adults.

(1) *Age of the rabbit.* To test the severity of the encephalitis following corneal inoculation three groups of young animals in individual litters were used.

Group 1. Three young rabbits of the same litter weighing 600, 600, and 670 grams respectively and about ten weeks of age were inoculated on the scarified right cornea with pus from the eye of a rabbit inoculated 48 hours previously. One of these rabbits died on the seventh day, another on the eighth, another on the ninth day afterward with herpetic encephalitis. The protocol of one will serve to illustrate the group.

R. 106. Wt. 670 gms.

11/24/23. Temp. 103.8° F. Inoculated on right cornea with pus from eye of R. 99.

11/25. Good take. Clear vesicles along lines of incision. Some pus.

11/26. Temp. 104°. Purulent kerato-conjunctivitis.

11/27. " 103.6°. Eye closed with purulent exudate. No turning of head.

11/28. " 105.9°. Head beginning to turn to right.

11/29. " 106.4°. Strong turning to right.

11/30. " 104.5°. Same.

12/1. " 105.4°. Beginning loss of equilibrium, falling to right.

12/2. Lying on right side moribund, grinding teeth.

12/3. Found dead.

With the same virus and at the same time another group was inoculated similarly.

Group 2. Four young rabbits weighing 240 grams each and about four weeks of age were inoculated on the right cornea after extensive scarification, with pus from the eye of R. 99 inoculated 48 hours previously. One of these died of herpetic encephalitis nine days later, the other three acquired an encephalitis but recovered completely. The protocol of one recovered animal is appended.

R. 109. Wt. 240 gms.

11/24/23. Right cornea inoculated.
11/25. Good take. Clear vesicles along incisions.
11/26. Temp. 103.8° F. Purulent kerato-conjunctivitis.
11/27. " 105.8°. Eye closed. Head turning to right.
11/28. " 105.4°. Strong turning to right.
11/29. " 105.2°. Same.
11/30. " 105°. Same.
12/1. " 105.5°. Same.
12/2. " 104°. Seems better. Less turning of head.
12/3. " 102.5°. No turning. Better.
12/4. " 104. 5°. No turning.
12/6. " 103.6°. Salivated. No turning.
12/14. " 103.8°. Completely recovered. Right cornea completely opaque.

An adult rabbit inoculated on the right cornea at the same time as a control for these two groups died on the seventh day after inoculation with typical encephalitis.

Group 3. Seven young rabbits twenty-six days old and averaging 250 grams in weight belonging to the same litter were inoculated on the right cornea with pus from a herpetic keratitis inoculated 48 hours previously. Each of these rabbits contracted a mild encephalitis, and all of them recovered. The following is a typical protocol.

R. 201. Young rabbit 26 days old. Wt. 250 gms.

3/7/24. Right cornea inoculated with pus from right eye of R. 192.
3/8. Temp. 102.7° F. Good take. Clear vesicles.
3/10. " 104.3°. Purulent kerato-conjunctivitis.
3/11. " 106°. Head turning to right.
3/12. " 105.8°. Little turn to right.
3/13. " 103°. No turn observed.
3/14. " 106.3°. Strong turn to right.
3/15. " 105. 7°. Same.
3/17. " 103.5°. Better. No turn.
3/18. " 103.4°. Salivation. No turn.
3/20. " 103.5°.
4/1. Complete recovery. Right cornea opaque.

A control adult rabbit inoculated on the right cornea with the same virus at the same time died nine days later with typical herpetic encephalitis.

These experiments demonstrate that young rabbits up to four weeks of age may acquire an encephalitis from an experimental corneal infection with herpetic virus, but usually in a mild form from which they rarely die. Our experience in this respect is contrary to

that of Ford and Amoss [2] with herpetic infections. They state that young rabbits are more susceptible to infection with herpetic virus than are adults. Adult rabbits, however, have in our hands proved so uniformly susceptible to herpetic encephalitis that in testing the virulence of a certain strain or in attempting to demonstrate the presence of herpetic virus in human or other material, we would not hesitate to use full-grown rabbits in preference to younger ones.

One small group of rabbits of a particular strain have proved to be very resistant to herpetic encephalitis, although they readily contract an infection of the brain following corneal inoculation. These rabbits were bought in a single lot and probably were of the same family.

(2) *Resistant strain of rabbits.* In this lot there were four rather large adult uniformly light brown rabbits of apparently the same size and age. One of them was inoculated upon the right cornea for passage in the usual way with virus in the pus from a 48 hour infection of the eye. An intense purulent kerato-conjunctivitis resulted and was as usual followed by a turning of the head to the inoculated side and a complete loss of equilibrium. The animal remained very sick for a few days, finally recovering. The remaining three rabbits of the lot, and also controls, were similarly inoculated. The controls died in the usual time, but each of the brown rabbits recovered. The following experiment illustrates the group.

R. 244. Non-pregnant large brown female.

4/3/24. Right eye inoculated with pus from eye of R. 240 (48 hour virus).
4/4. Temp. 103° F. Good take on cornea.
4/5. " 103.5°.
4/7. " 105.3°. Head turning to right.
4/8. " 105.8°. Same.
4/9. " 105.1°. Beginning loss of equilibrium.
4/10. " 107.3°. Complete loss of equilibrium. Lying on right side struggling.
4/11. Sitting up in cage, very weak, but recovering.
4/12. Temp. 105°. Same.
4/17. " 105.5°. Unsteady, head drawn to right. Gnashes teeth. Nystagmus left eye.
5/1. Has steadily improved. Impairment of equilibrium.
6/14. Has recovered, but is still unsteady with tendency to turn head to right. Temp. 102.5°.

Two adult rabbits similarly inoculated at the same time served as controls. Both died eight days later with herpetic encephalitis.

While this is a small group of animals from which to draw the inference that a certain breed of rabbits is more resistant to herpetic encephalitis than others, the uniformity of our results with many rabbits of various breeds and mixtures has been, in our opinion, of sufficient constancy to justify this conclusion.

Summary

1. The incidence and severity of herpetic encephalitis resulting from a peripheral focus of infection in rabbits depends upon the virulence, i.e., the "neurotropic" property of the strain of virus, and the size of the peripheral area infected.

2. Rabbits highly susceptible to herpetic encephalitis may be immunized against this infection by inoculating a sufficiently small area of the cornea with a highly neurotropic strain without causing encephalitis.

3. The severity of an encephalitis depends among other things upon the quantity of virus entering the brain through a peripheral nerve.

4. Very young rabbits are strongly resistant to herpetic encephalitis and rarely die following an infection of the brain through a herpetic keratitis.

5. A certain breed of rabbits has been observed which has a stronger natural resistance to herpetic encephalitis than others.

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RIEDEL'S STRUMA ASSOCIATED WITH REMNANTS OF THE POST-BRANCHIAL BODY *

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Enlargement of the thyroid gland due to chronic inflammation is uncommon and when it does occur the preoperative diagnosis is rarely correct. Goitre of this type was first described by Riedel in 1896 and is still designated as Riedel's iron-hard struma.

The subject of the present paper was a man, fifty years old, admitted to the surgical service of Dr. T. H. Russell. The patient complained only of a swelling in the neck, failing eyesight and hoarseness. Family history and earlier personal history were unimportant. The enlargement of the neck had been observed four or five months before and it had gradually increased, without pain. It obviously involved both lobes of the thyroid gland and it was extremely hard. The tonsils were enlarged and there was a severe inflammation of the pharynx and trachea. The preoperative diagnosis was carcinoma.

At operation the tumor mass was removed entire without great difficulty. Postoperative recovery was uneventful.

Two months later the patient's tonsils were removed by Dr. M. F. Jones. One tonsil measured 31 × 20 × 14 mm., the other 21 × 15 × 12 mm. The tonsils showed an increase of lymphoid tissue, irregular fibrosis, thickened septa and capsule. Leptothrix colonies were present in the crypts. In the dense fibrous tissue attached to the capsule there was a large blood vessel filled with an organized thrombus.

Recent reports indicate that the patient has fully recovered his health.

The surgical specimen from the first operation (Fig. 1) presented an almost symmetrical enlargement of both lobes of the thyroid gland and measured 19 × 6 × 4 cm. The external surface presented rounded prominences but was smooth except for delicate fibrous tabs. The capsule was thickened and in it there were numerous con-

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gested vessels. The specimen was very hard and, on section, the cut surface everywhere presented the same appearance, yellowish-white with thin translucent lines between small irregular opaque areas, somewhat suggesting lobules. There was no visible colloid. A mass of lymph nodes, 18 × 7 × 5 mm., was attached to the middle of the anterior, inferior border. These nodes were firm and on section light pink with small hemorrhagic spots.

The histologic structure in no way suggested carcinoma but rather was that of a chronic granuloma. Dr. James Ewing, to whom microscopic specimens were submitted, recognized the condition as a fairly late stage of Riedel's struma. His counsel and stimulating encouragement are gratefully acknowledged.

Sections from all parts of the specimen show a striking change in the thyroid structure (Fig. 2). The capsule is thickened and consists of thin irregular lamellae of fibrous tissue. In some places it contains collections of round cells and plasma cells, in the center of which may be seen a small blood vessel, often with swollen endothelium. A few of these contain polymorphonuclear leucocytes. Wide septa of fibrous tissue extend inward from the capsule into the substance of the gland and fascicles of fibrous tissue cross and recross the intervals between the larger bands in every direction. The fibrous tissue is in part hyaline and structureless and in part composed of fibers between which can be seen the elongated nuclei of the connective-tissue cells. At other places one finds younger fibroblastic cells with long protoplasmic processes. Scattered through this fibrous tissue there are both diffuse and circumscribed collections of small lymphocytes and plasma cells, scattered well-defined lymph follicles and, in widely separated areas, remnants of gland vesicles.

The thyroid parenchyma persists especially just beneath the capsule but is also to be found at various depths (Fig. 3). In these areas one finds thyroid vesicles compactly grouped to form lobules 3 to 10 mm. in diameter. Many of these contain colloid. Lymph nodules encroach upon the lobules and an exudate of lymphocytes and plasma cells is found within the lobule, about the vesicles, between the epithelial cells and also in the lumina. In many places the epithelial lining of the vesicle has disappeared entirely or in part, being replaced by fibrous stroma containing inflammatory cells. This intralobular fibrous tissue is at times so abundant and compact as to be with difficulty distinguished from the interlobular bands.

In other locations the parenchyma persists as isolated groups of epithelial cells embedded in the fibrous tissue without any arrangement suggesting thyroid lobules (Figs. 4, 5 and 6). These isolated portions of epithelium sometimes surround colloid or they may appear as irregular clumps or cellular strands. The individual epithelial cells are well preserved and in them mitotic division figures are occasionally found (Fig. 6).

The well defined lymph nodules are most abundant near the capsule but they are found throughout the substance of the specimen, at least one and often several in each square centimeter of tissue surface. These follicles are especially conspicuous at the margins of the recognizable thyroid lobules (Fig. 3). Such a follicle is from 100 to 900 μ in diameter with lymphocytes at the center and abundant plasma cells in the peripheral zone. Active germinal centers are lacking.

The blood supply is plentiful but is not a striking feature in the pathological picture. Occasionally a vessel with thickened wall is seen.

Rarely in some of the sections peculiar groups of epithelial cells are encountered (Figs. 7 and 8). These cell nests are prominent features and appear to have persisted with only slight change in the midst of the inflammatory transformation occurring in the surrounding thyroid tissue. Such a nest may be found in a lymph nodule (Fig. 7), in an interlobular septum or within the thyroid lobule. As a rule the nest is solitary (Fig. 7) but they may be paired or several may be found in one microscopic field. Several closely related groups of these nests are found just beneath the anterior capsule near the central zone of each lateral lobe (Fig. 8). The nests vary in shape from round and oval masses to irregularly branched groups with thick blunt prolongations. They measure 30 to 200 μ in diameter. The epithelial cells are several rows in depth and the outer row rests upon a definite basement membrane. There is, however, no evident fibrous capsule. These epithelial cells are large and polyhedral with oval vesicular nuclei. In some places the cells are separated by narrow gaps traversed by fine intercellular bridges, suggesting the lymph-canalicular system of squamous epithelium (Fig. 8). When compressed the cells appear flattened and curved about each other (Fig. 7).

A few of these cell nests show lumina, lined by cuboidal or cylin-

drical cells. One larger nest with a lumen appears to possess a duct leading away from it (Fig. 9).

These peculiar cell nests are interpreted as remnants of the post-branchial body, described by Getzowa. Here are found the solid cell nests representing the glandular parenchyma of the post-branchial body (Figs. 7 and 8) and the hollow cell nests with ducts representing the accessory cysts (Fig. 9). The main duct representing the original pharyngeal pouch has not been found.

The post-branchial, or more exactly the ultimo-branchial body, is rarely found in human material. Hermann and Verdun in a study of many human embryos recognized this structure within the thyroid gland in only three, 55, 63 and 95 mm. in length, respectively. They never found it in the adult thyroid.

Getzowa demonstrated the ultimo-branchial body in seven individuals, one an embryo 90 mm. long, another a new-born infant, third, an infant three weeks old, and in four adults (three cretins and one idiot). Pathological overgrowth of these remnants has been described by Getzowa and by Langhans. Berard more recently has reported a branchioma possibly originating in these rudiments.

The possible relation between these post-branchial remnants and Riedel's struma appears not to have been considered.

Riedel waited twelve years before reporting his first iron-hard struma, on account of its unique and inexplicable features. At the end of this period he observed a second similar goitre. Almost immediately Cordua, Tailhefer, Berry and Ricard reported similar cases. These early reports deal chiefly with clinical findings with only scant attention to the structural changes. As a rule the patients had been well until there appeared a painless swelling of the thyroid gland which progressed until the symptoms due to pressure and adhesions compelled them to seek relief.

Silatchek, in 1910, under the title "Peristrumitis Indurativa," added another case and also described the pathological picture in some detail. A report by Spannaus appeared in the same year. Delore and Alamartine, in 1911, reported a new case and collected thirteen in the literature. There quickly followed, in 1912, a report by Murray and a report of four cases observed by Hashimoto. Hashimoto regarded his disease as distinct from Riedel's struma and named it "Struma Lymphomatosa." Later observers, notably Ewing, consider the microscopic findings of Hashimoto to represent

TABLE I. *Reported Cases of Riedel's Struma without concomitant Tuberculosis or Syphilis*

No.	Author	Year	Sex and Age	Preoperative diagnosis	Duration	Lobe	Remarks	Operation	Sequel
1	Riedel	1894	M 40	Sarcoma	?	Both	—	Partial resection	Recovery
2	Riedel	1894	F 4	Strumitis	?	Both	—	Partial resection	Recovery
3	Riedel	1896	M 42	Carcinoma	6 mos.	Both	Goitre 1 yr.	Inoperable	Death in 15 mos.
4	Riedel	1896	F 23	Strumitis	2 mos.	Both	Mother had goitre	Inoperable	Death in 2 mos.
5	Riedel	1897	M 29	Fibrosarcoma	2 mos.	Right	KI ineffective	Partial resections (2)	Recovery 10 yrs. later
6	Cordua	1896	F 13	Sarcoma	3 yrs.	Right	—	Inoperable	Recovery
7	Tailhefer	1897	M 20	Carcinoma	3 mos.	Left	—	Small resection	R. hemiplegia
8	Berry	1898	F 40	Carcinoma?	5 wks.	Right	Nodular	R. lobe removed	Recovery
9	Ricard	1901	M — †	Carcinoma	?	Both	—	Total extirpation; 4th nerve cut	Fistula of thoracic duct. Recovery
10	Silatchek	1910	M 32	Peristrumitis indurativa	4 yrs.	Right	Father had goitre; KI ineffective	Exploratory incision X-Ray	Tardy recovery
11	Spannaus	1910	M 52	Carcinoma	18 mos.	Both	Goitre 7 yrs; KI ineffective	Partial resection	Recovery
12	Delore and Alamartine	1911	M 29	Carcinoma? Thyroidite ligneuse	2 mos.	Right	Goitre 1 yr.	Resection; jugular vein cut	Death in 4 days
13	Murray	1912	M 23	Carcinoma?	3 mos.	Both	Goitre 18 mos.	Partial resection	Recovery; myxedema *
14	Hashimoto	1912	F 61	Parenchymatous goitre	7 mos.	Both	Good health	Partial resection	Recovery; myxedema *
15	Hashimoto	1912	F 40	Carcinoma?	6 wks.	Both	Good health	Partial resection	Recovery; myxedema *
16	Hashimoto	1912	F 55	Struma fibrosa	4 wks.	Both	Good health	Partial resection	Recovery; myxedema *
17	Hashimoto	1912	F 45	Struma fibrosa	?	Both	Good health	Partial resection	Recurrent goitre
18	Meyer	1913	F 25	Malignancy	9 mos.	Right	Goitre; KI ineffective	Partial resections (2)	Pneumothorax; death
19	Heinike	1914	F 50	Carcinoma	1 yr.	Both	KI and arsphenamine	Complete extirpation	Myxedema *
20	Heinike	1914	F 47	Carcinoma	1 yr.	Both	Goitre 2 yrs.	Partial resection	Recovery; X-Ray
21	Reist	1922	F 40	Carcinoma?	Brief	Both	Good health	Removal	Recovery; myxedema *
22	Reist	1922	F 41	Carcinoma?	?	?	Good health	Removal	Recovery
23	Reist	1922	F 50	Sarcoma?	1 yr.	Right	Goitre 5 yrs.	Removal	Recovery
24	Reist	1922	F 60	Carcinoma?	4 mos.	Left	—	Removal	Recovery after 1 yr.
25	Meeker	1923	M 50	Carcinoma	5 mos.	Both	Good health	Removal	Myxedema slight

* Myxedema following thyroidectomy was successfully treated with thyroid.

† A young person, age not recorded.

an early stage of Riedel's iron-hard struma. In 1913, Meyer reported a malignant granuloma of the thyroid gland, which was later classified as Riedel's struma. In 1914, Heinike reviewed the literature under the title, "Chronic Thyroiditis." In the same year, Monod in a Paris thesis reported three cases of Riedel's struma which he believed to be due to syphilis.

The only references to Riedel's struma found in the American literature are in Ewing's "Neoplastic Diseases," Crotti's "Thyroid and Thymus" and Marine's monograph on the thyroid in "Endocrinology and Metabolism." [1]

Among the outstanding features of this disease may be mentioned first the hardness of the thyroid mass, compared to iron, cartilage, bone and wood by various authors. The size has varied from that of an apricot to the mass shown in Fig. 1. Some authors, Spannaus, Delore and Alamartine, have claimed that goitre always precedes Riedel's struma but this is denied by Heinike. According to the reported observations the disease may appear in previously normal glands, in diffusely enlarged thyroids or in adenomatous nodules within the thyroid.

Riedel in 1896 and again in 1911 advocated partial resection. Ricard in 1901 removed the total mass for the first time. Ewing, 1922, advocates partial resection and radiation. Retrogression after partial extirpation has been a striking phenomenon of this disease and recovery after operation has been the rule. The administration of iodine has been without effect.

Post-operative myxedema, partial paralysis and operative death have occurred. In general the results of the more radical operations, such as attempted complete resection in the face of extensive adhesions, have been poor.

The general features of the reported cases are summarized briefly in Table 1.

The histologic findings in all cases agree for the most part in their essential details with those described by Riedel. The later authors have described the microscopic picture more minutely. A few authors, Murray, Hashimoto and Meyer, have found peculiar variations in structure but all agree that the condition is essentially a chronic inflammation.

[1] To these may be added the recent paper by A. W. St. George "Chronic Productive Thyroiditis." Annals of Surgery, July, 1924.

TABLE 2. *Reported Observations of Post-branchial Bodies in Human Subjects*

No.	Author	Year	Sex	Age	Condition	Thyroid	Post-branchial remnants			Location in thyroid, lobe
							Paren-chyma	Accessory cysts	Main cyst	
1	Hermann and Verdun	1899	F	55 mos.	Embryo	Normal	+	+	–	Right
2	Hermann and Verdun	1899	M	63 mos.	Embryo	Normal	+	+	+	Both
3	Hermann and Verdun	1899	M	95 mos.	Embryo	Normal	+	+	+	Both
4	Getzowa	1907	M	56 yrs.	Cretin	Atrophic	+	+	+	Both
5	Getzowa	1907	M	47 yrs.	Cretin	Atrophic	+	–	–	Left
6	Getzowa	1907	M	56 yrs.	Cretin	Atrophic	+*	–	–	Both
7	Getzowa	1907	M	50 yrs.	Idiot	Atrophic	+	+ left	+ left	Both
8	Getzowa	1911	F	3 wks.	—	Athyriosis	+	+	+	?
9	Getzowa	1911	M	New-born	—	Normal	+	+	+	?
10	Getzowa	1911	?	90 mos.	Embryo	Normal.	+	+ right	+ right	Both
11	Meeker	1923	M	50 yrs.	—	Riedel's struma	+	+	–	Both

* A benign tumor nodule developed from the post-branchial body in this instance.

In attempting to elucidate the peculiar pathology of Riedel's struma various authors have related it to Basedow's disease. Others have compared it to Riedel's pancreatitis, to Mikulicz's lacrymal granuloma, to Kuettner's disease of the salivary gland and to contracted kidney. To such a comparison there has been raised the objection that these glands possess open excretory ducts while the thyroid does not.

The presence of remnants of the ultimo-branchial body in the present instance is of peculiar interest, for should such remnants prove to be an essential feature of Riedel's struma, their presence would serve to relate this disease to the chronic inflammations of the glands just mentioned. Furthermore the peculiar pathology and rare incidence of the disease might then be explained as a result of extension of inflammation from the pharynx or trachea along the ultimo-branchial duct system, or its accompanying lymphatics, into the thyroid gland, an extremely rare phenomenon because this rudimentary duct system rarely persists even at birth (Table 2).

Summary

1. Riedel's struma is a chronic inflammation of relatively rare occurrence in the thyroid gland. It presents clinical manifestations very similar to those of malignant neoplasms in this organ, but is distinguished by its benign course when the pressure is relieved.

2. Recovery may be expected after partial extirpation or radiation. Iodine feeding is not beneficial. Total extirpation is frequently followed by myxedema.

3. Anatomically the disease is characterized in its early stage by hyperplasia of the thyroid parenchyma and later by an infiltration by lymphocytes, plasma cells and eosinophilic leucocytes with slight inflammatory manifestations on the part of the blood vessels. True lymph follicles are abundant in the early stages. As a result there is extensive degeneration of the parenchyma and proliferation of fibrous tissue which is remarkably abundant in the late stages. The inflammation may extend far beyond the limits of the gland.

4. The tonsils and peritonsillar tissue show a similar chronic inflammation and emphasize the pharyngeal involvement. The adjacent lymph nodes show chronic inflammation.

5. A specific causative agent has not been recognized.

6. Regenerative activity on the part of the parenchyma and persistent cell nests and accessory cysts representing the post-branchial body are observed for the first time in this case.

7. From these findings it is not unreasonable to assume that a primary pharyngitis or tracheitis may extend by way of the post-branchial system into the thyroid.

8. Since post-branchial remnants are found in human embryos, in athyriosis and in cretins and have not been found in normal adult thyroid glands, it is suggested that thyroids containing such rudiments may be of low vitality and quickly reach the stage of exhaustion and may, therefore, be peculiarly susceptible to the extreme form of atrophy and fibrous replacement seen in Riedel's struma.

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DESCRIPTION OF PLATES V–X

Fig. 1. Photograph of gross specimen.

Fig. 2. Low-power photomicrograph of a typical area. There is extensive fibrosis and diffuse infiltration by lymphocytes and plasma cells; a lymph follicle near the bottom.

Fig. 3. Higher magnification of an area showing lobule of thyroid gland. (1) Thyroid vesicles. (2) Lymphocytes invading vesicles and actually replacing the degenerated epithelium. (3) Lymph follicle.

Fig. 4. High-power drawing showing the fate of the thyroid epithelium. (1) Vesicle with colloid. (2) Degenerating vesicle. (3) Epithelial remnants of vesicle.

Fig. 5. Field adjacent to that shown in Fig. 4. (1) Epithelial remnants of thyroid vesicles. (2) Plasma cells. (3) Small lymphocytes.

Fig. 6. Another field adjacent to that shown in Fig. 4. (1) Thyroid epithelium. (2) Epithelial cell in mitosis.

Fig. 7. High-power drawing. (1) An epithelial cell nest representing the post-branchial body. (2) Remnants of thyroid epithelium. The epithelial nest is within a lymph follicle.

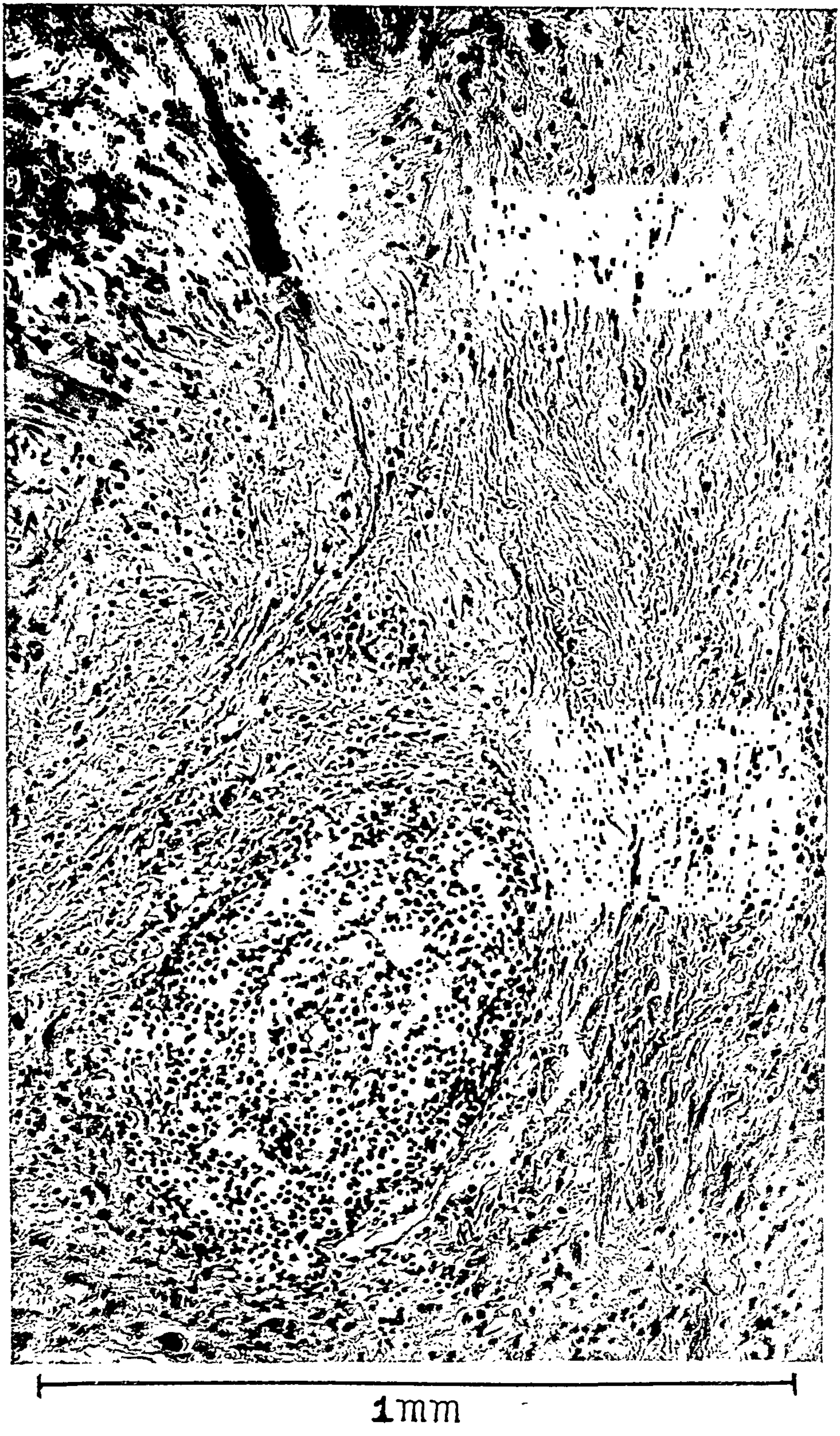
Fig. 8. High-power drawing of a group of solid cell nests similar to that shown in Fig. 7, found under the anterior capsule in lateral lobe. (1) Branching end buds of the parenchyma of the post-branchial body. (2) Cell nest with lumen lined by cuboidal cells.

Fig. 9. High-power drawing of a group of epithelial cells representing an accessory cyst of the post-branchial body. (1) Wall of cyst. (2) Cavity of cyst. (3) Duct of cyst.

Fig. 10. Illustration (from Getzowa's paper) of an accessory cyst for comparison with Fig. 9. (1) Wall of cyst. (2) Cavity and duct of cyst. (*After Getzowa.*)

1

Meeker Riedel's struma



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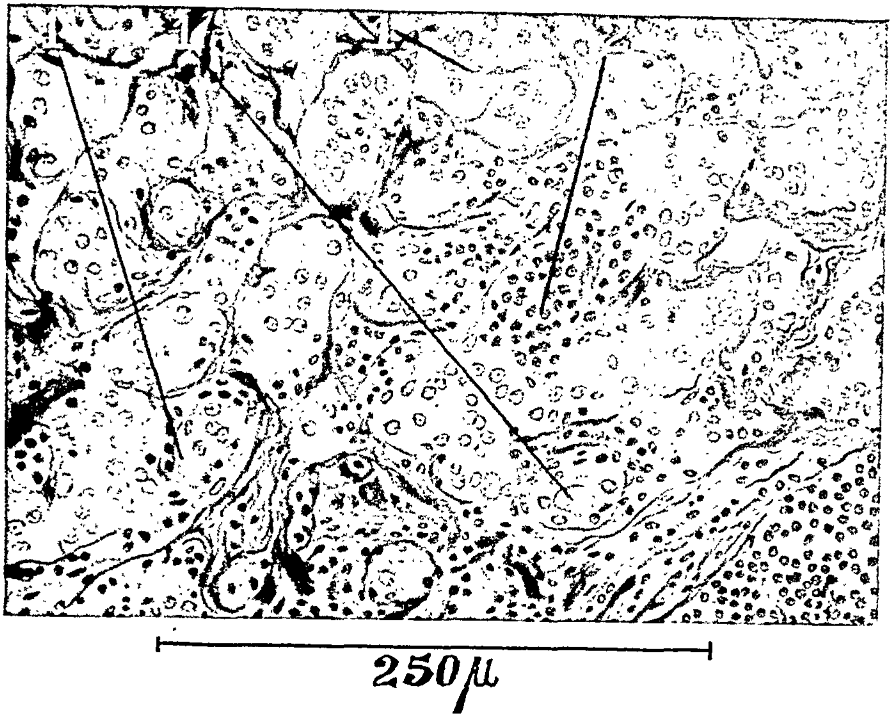

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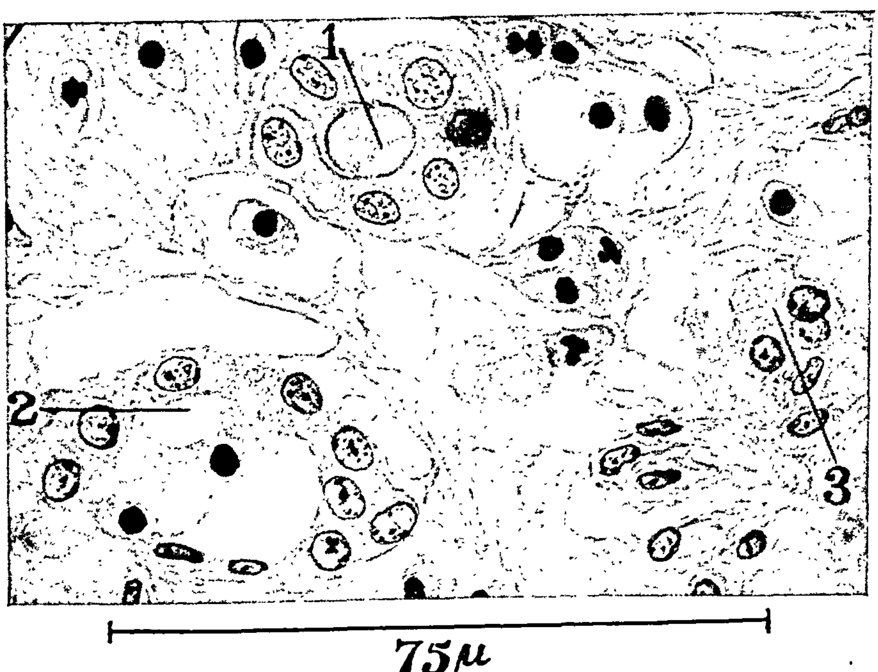

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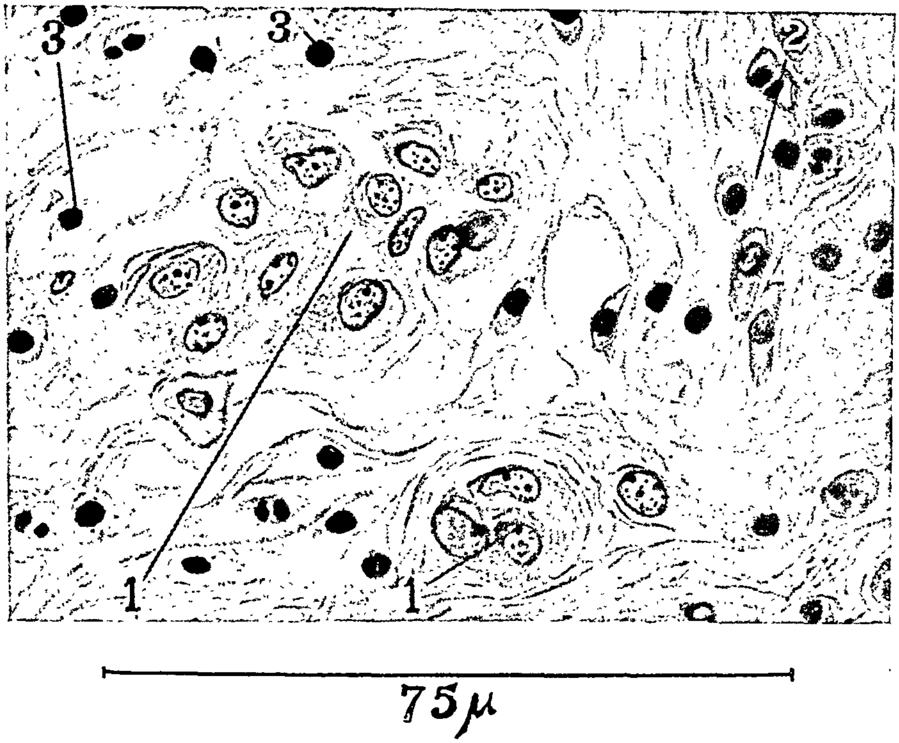

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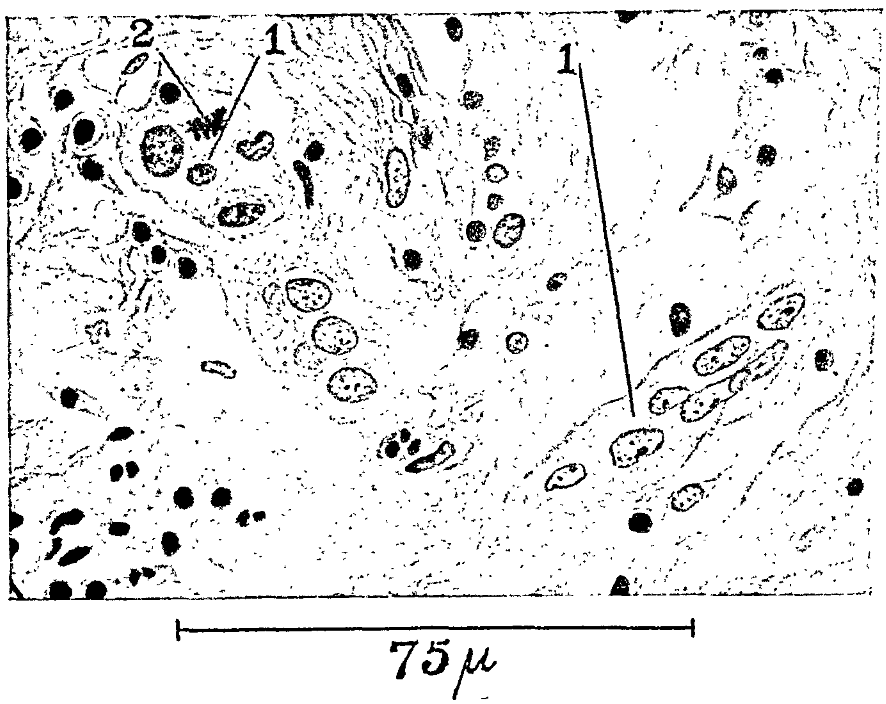

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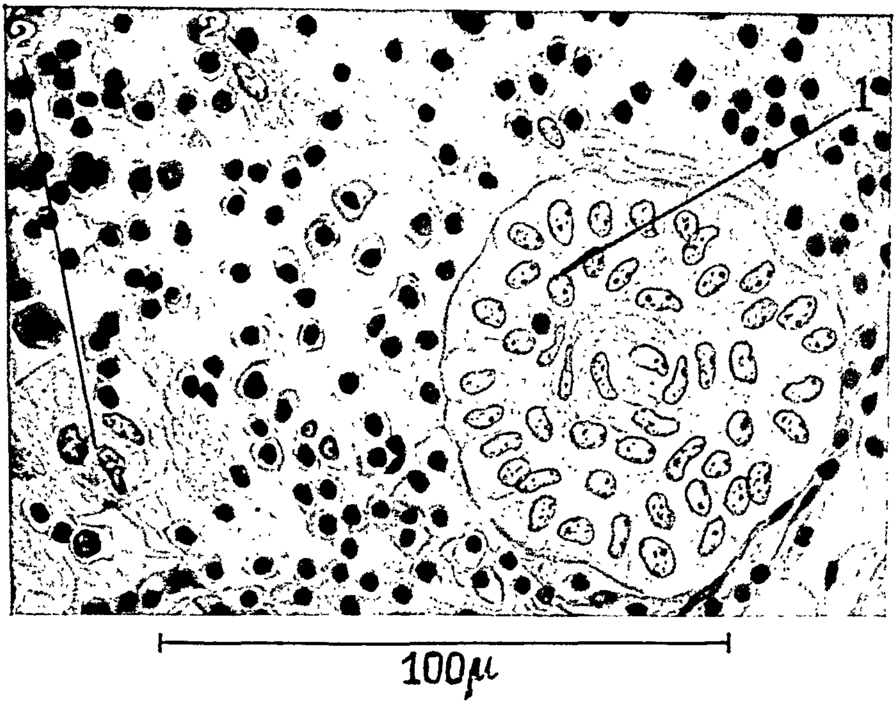

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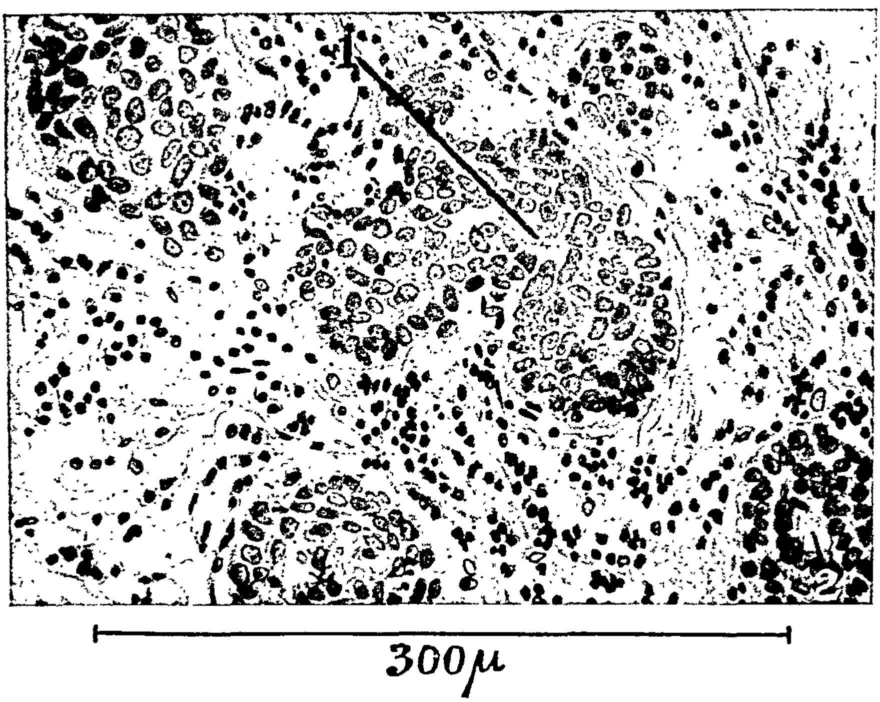

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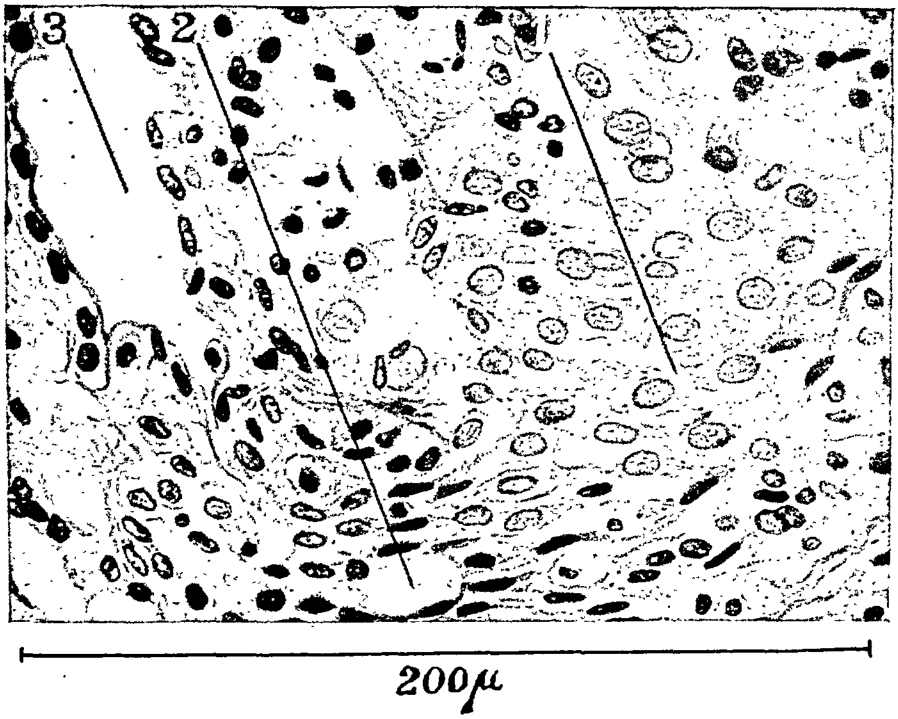

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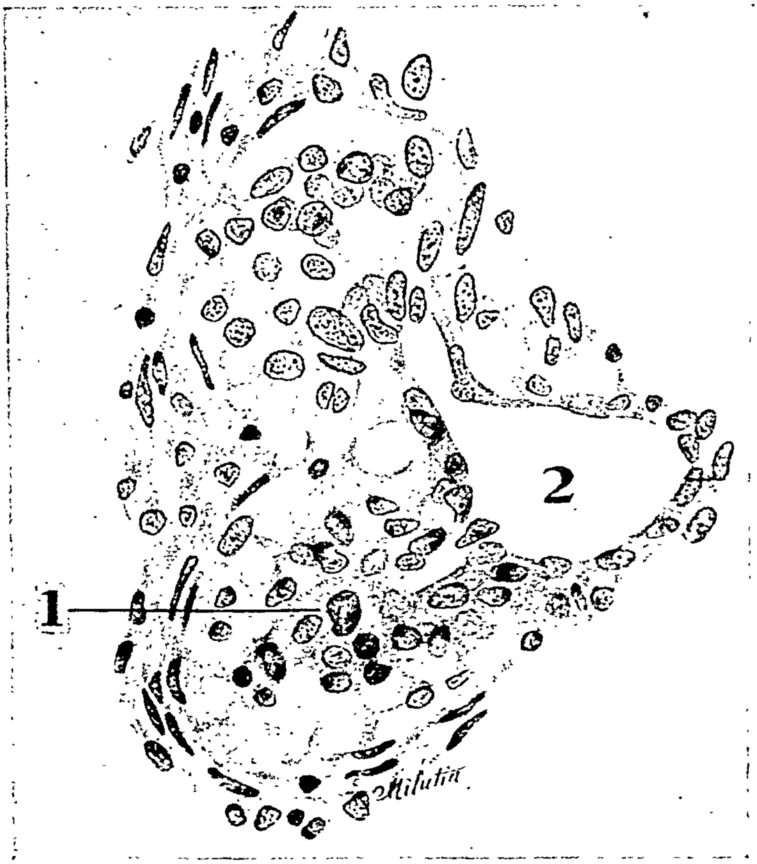

10

HETEROTOPIA OF THE BONE MARROW WITHOUT APPARENT CAUSE *

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The occurrence of tissue resembling bone marrow in positions other than in the bony cavities is uncommon and usually is found associated with bone, as a result of chronic inflammation, or appearing in some other organ of the hematopoietic system, as the result of a need for more blood cells in the circulation. The present case, however, is much rarer in that apparently normal (slightly hyperplastic) marrow was found in bilaterally symmetrical nodules, unassociated with bone, in a case in which the blood cell forming mechanism was apparently adequate.

Definitions

To clarify the meaning of certain terms used in this article, the following terms (which I have not been able to find grouped together elsewhere) are defined in accordance with accepted authorities.

Metaplasia is "The postnatal production of specialized tissues from cells which normally produce tissues of other orders and is an adaptation on the part of the cells to an altered environment" . . . "Yet metaplasia is bounded by rigid laws; epithelial tissue can only be converted into other forms of epithelial tissue, mesoblastic tissue only into forms of mesoblastic" (Adami and McCrae).[1]

In *heteroplasia*, "There is no conversion of one type of tissue into another, but there is merely a persistence of characters and cell relationship peculiar to an earlier period of growth."

Heterotopia "May be congenital or acquired and consists of the abnormal snaring of cells or organs from the organ proper, and then subsequent growth in another place." In the present article there is no implication intended as to how the tissue occurred in its abnormal site.

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Ectopia, though originally used more or less synonymously with heterotopia, is better reserved to apply to the misplacement of organs, usually congenitally, to neighboring regions.

Undifferentiation ("histological accommodation" of Aschoff) [2] is the loss of differential characters by cells which have become subject to abnormal conditions, for example, the flattening of cylindrical cyst epithelium through mechanical pressure.

Autochthonous growth occurs when cells, or tissue, abnormal in location are derived from cells formed in situ.

In contradistinction, *colonization* is the growth of tissue in an abnormal environment, originated by cells derived from distant points.

Heterotopia of the Bone Marrow

True bone and bone marrow are not infrequently associated together in metaplastic bone formation. This phenomenon, which usually is due to a long standing, chronic inflammation, has been found in the lungs, pleura, Fallopian tubes, kidneys, arteries and lymph nodes. An instance of such an occurrence in the lungs, which is the most common site of this phenomenon, was recently reported by Dr. M. Strumia from the Philadelphia General Hospital. The frequent occurrence of bone and bone marrow metaplasia in the hilum of the kidney is of interest in connection with Jordan's[3] recent report that this is one of the chief sites of blood formation in the frog's larva.

Bone marrow with normal cellular structure (i.e., with arteries, veins, fat and reticulum, as well as with the various appropriate blood cells) has rarely been noted in other parts of the body than the medullary cavities of the bone without association with bone. Some cases illustrating such a development of bone marrow without bone are given below, without touching on the rather extensive literature of myeloid metaplasia of the spleen (see Tanaka [4]). As a member of the hematopoietic system it is natural that the spleen should more easily react to this activity. Excepting in this organ I could find reference to only five cases in which it was not associated with either leukemia or pseudoleukemic anemia, though the literature on the subject was carefully searched.

In 1905 Gierke [5] reported a case in which bone marrow structure was found in the adrenal. The important associated lesions were

cardiac with hemorrhagic infarcts in the lung. He stated that Heidelberg had noted a somewhat similar case before and his was the second one of its kind.

In 1909 M'Kenzie, Browning and Dunn [6] recorded two cases in which they found typical bone marrow structure in the hilum of each kidney. The first case, aged 9 months, had massive confluent bronchopneumonia and a leukocyte count of 90,000 with many erythroblasts and other abnormal cells in the blood. The other case, 20 months old, had severe rickets. In the former case the bone marrow masses were present in the hilum around the vessels with some myelocytic proliferation throughout the whole kidney substance. The spleen and lymphatic glands were enlarged and showed myelogenic changes; the bone marrow showed evidence of active proliferation. In the rickets case the bone marrow masses were in the hilum and the kidney substance was not infiltrated. The bone marrow in the ribs was almost entirely replaced by osteoid tissue and fibrotic proliferation. The spleen was enlarged and showed evidence of myelogenic activity.

In 1912 Tanaka [4] reported two cases in which the bone marrow tissue was present in the hilum and substance of the kidney. One was 9 months old and the other 2 years old. Both of them had "splenic anemia" and severe rickets.

In 1918 Matsunaga [7] and in 1919 Mieremet [8] each reported a case of heterotopia of the bone marrow. In the former it was present in the hilum of the kidney, the patient having had myelogenous leukemia with a leukocyte count of 144,000. In the latter case it was present in the adrenal, the patient having had carcinoma of the esophagus and terminal aspiration bronchopneumonia.

In 1922 Herzenberg [9] described three cases. In two the heterotopic bone marrow was present in the pelvis of the kidney; in both instances the patient was 2 years of age and had splenic anemia. In the third case it was found in an accessory adrenal, in a patient aged 69 years who died from cardiovascular disease.

CASE REPORT

The case the author wishes to present apparently differs in several respects from any hitherto recorded. So far as can be determined by carefully reviewing the literature and by personal communication with various pathologists (Drs. A. J. Smith, McFarland, H. Fox,

Ewing, Krumbhaar, Sabin and Lucke) it is the first one of its kind noted. The protocol in brief is as follows:

Clinical History: L. B., white female, aged 81 years, admitted to the Philadelphia General Hospital Feb. 18, 1924.

Family History, Negative.

Past History: Nothing of importance and nothing suggesting anemia.

Present Illness: She had an attack of hemiplegia 2 months ago and has been in bed ever since. Four weeks later she became irrational.

Physical examination revealed an obese white female with senile dementia, right hemiplegia, general arteriosclerosis, and hypertrophied heart with a systolic murmur; death occurred one week after admission from terminal bronchopneumonia.

Necropsy notes: Adult white female, height 155 cm., weight 135 lbs., autopsy performed 23 hours after death.

Heart, hypertrophied, weighing 570 gms.

Arteries, general arteriosclerosis.

Lungs, patches of bronchopneumonia.

Spleen enlarged, weighing 405 gms.

Liver, congested; gall bladder, filled with gall stones.

Kidneys and other organs showed arteriosclerosis.

Brain showed arteriosclerosis, multiple areas of thrombotic softening, pituitary adenoma.

On each side of the thorax at the fifth rib near the vertebral column a small, reddish, smooth, encapsulated, rounded and nodular mass was noted. Each one measured 2.5 cm. in diameter (Fig. 1). They were adherent to the ribs with fibrous tissue. After their removal the ribs beneath were smooth and showed no erosion or disturbance of the periosteum. No connection between them and the marrow in the medullary cavities could be determined. No similar masses were found anywhere else in the body. No bony disturbance was noted, although, as the true nature of these tumors was not suspected, no examination of the bone marrow was made. The cut surfaces of the two masses were alike, purplish red, moist, smooth, elastic and with a homogeneous appearance. The capsule was fibrous and thin. The whole mass looked like an organizing blood clot.

Microscopically several sections from each mass showed slightly hyperplastic normal bone marrow structure with some hemorrhage. The structure consisted of wide capillaries and between them the parenchymal cells — Myeloblasts, myelocytes, leukocytic eosinophiles, eosinophilic myelocytes, multinucleated giant cells, erythrocytes and erythroblasts, many fat cells, connective tissue cells and blood vessels occurring approximately in the proportion normally found in hyperplastic marrow (Figs. 2 and 3). There was much pigment both within the macrophages and outside them, especially at the margins of the tissue (Fig. 3). Sections from the various areas gave much the same picture. The oxydase stain confirmed the presence of the myelocytic cells (Fig. 5). The iron stain gave a positive Prussian blue reaction, showing that the pigment originated from the red cells.

DISCUSSION

This case apparently differs from any other reported in which bone marrow heterotopia has been found both in the location and symmetrical arrangement of the masses, and in their separation from any of the organs of the body; also it differs from most cases in not having any apparent cause. In the bone metaplasia cases, for instance, each was accompanied, or preceded, by an inflammatory process. In the other cases the heterotopic bone marrow structure was present in pathological conditions of the blood-forming organs except Mieremet's case, carcinoma of the esophagus, M'Kenzie's, Browning's and Dunn's case, severe rickets, two mentioned by Gierke, and Herzenberg's third case, cardiovascular disease. In these cases the adrenals, and the pelvis and the hilum of the kidney were the sites of this phenomenon and in all the hematopoietic system was more or less involved.

Why is this heterotopic bone marrow most commonly found in the adrenal and the hilum of the kidney? M'Kenzie, Browning and Dunn stated that examinations of fetuses 10 weeks to 9 months old showed, lying in the hilum of the kidney around the vessels, small masses of tissue which give evidence of hematopoietic activity. And in very early embryonic life these masses are situated in close relationship to the cortical layer of the adrenal. Maximoff is said to have produced experimentally bone and bone marrow in the pelvis of the kidney by ligating the vessels. Thus the location of bone and bone marrow in this region suggests either a persisting embryonic function, or a renewed activity due to some unknown stimulus, of a function which ceased after birth.

The hemorrhage and deposits of blood pigment, both striking features in all the sections, are probably due to the imperfect circulation in the heterotopic tissue which caused stagnation of the red cells after their formation.

The reason for the occurrence of heterotopic bone marrow is still in dispute. Two theories are offered: One group of investigators believes it is autochthonous, while others believe it is formed by colonization by emboli derived from the blood forming organs. According to Herzenberg the former theory is supported by Sternberg, Schridde and Maximoff; the latter by Ziegler and Ribbert. Tanaka considered the heterotopic bone marrow in the two cases he

reported to be of embolic origin. Pollack,[10] who with Lubarsch made an extensive study of myeloid metaplasia in the lymph nodes, believes in the autochthonous theory. Ewing[11] in discussing the heteroplastic deposits in leukemia seems to be in favor of the embolic origin. MacCallum[12] after discussing the origin of the myeloid foci in the distant organs says, "To me the idea of transplantation of cells seems more plausible."

Gierke, who found heterotopic bone marrow in the adrenal, believed this to be metaplasia from local cells, and considered it tumor formation having normal bone marrow elements. These cells he thought were brought there during embryonic life and later developed.

Mieremet and Herzenberg agree with Gierke and consider their cases to be of autochthonous origin from congenitally misplaced cells.

Those that support the colonization theory are principally the authors who found the bone marrow tissue in the pelvis and the hilum of the kidney in cases of leukemia and pseudoleukemic anemia. On the other hand those that support the autochthonous theory are the authors who report heterotopic bone marrow tissue in the adrenals. They consider the misplacement of the bone marrow cells to have occurred in the embryo with the migration of the sympathetic tissue to form the medullary structure.

In the case under consideration it is hard to conceive a way in which the colonization could have occurred, because so far as is known no disease of the blood forming organs was present. Therefore it more likely originated autochthonously by metaplasia or developed from congenitally misplaced bone marrow cells. In view of the symmetrical arrangement the latter is more probable. The majority of those with whom I have discussed this case support this view, while Dr. Ewing, who does not believe they are of embolic nature, thinks it is compensatory effort on the part of nature to supply the necessary amount of blood cells. But it is hard to believe it compensatory, even though the rest of the bone marrow was not examined, in view of the absence of any evidence of a demand for an excess of blood cells. No one to whom it has been shown thinks it is a tumor in the sense of an ordinary myeloma.

It is necessary, however, to consider the possibility of the true neoplastic nature of these tumors. If this is granted, a type of

benign myeloma must be conceded in which all the elements of the bone marrow are present and in approximately normal proportions.

An interesting comparison with this case is here suggested by the finding in another case recently studied in these laboratories (antemortem report by Young and Cooperman [13]). In a case of osteitis fibrosa cystica autopsied in June, 1922 (No. 6824), the typical bony changes of this disease were found in the pelvis, humerus, vertebrae, ribs, etc. In the thoracic cavity arising from the parietal pleura a large rounded mass, reddish and measuring about 6 cms. in diameter, was noted. Though adherent to lung substance with fleshy bands and spicules of bone, it had probably arisen from the rib. The microscopical sections, which were taken several centimeters away from the rib, showed a typical hyperplastic bone marrow structure containing the various cellular elements previously mentioned, with the exception of the giant cells which were a predominant feature in the sections of the bone marrow proper. This histological difference, together with the extensive destruction of bone marrow through the body suggests that in this case, we are dealing with a compensatory effort (metaplasia from connective tissue), though it is not possible to rule out that this may be a myeloma of mixed cell type having a metaplastic origin. If this is considered to have arisen in adult life from misplaced bone marrow anlage, it could properly be considered a neoplasm, though essentially differing from the previous explanation only in the time element; if the development occurred in embryo then the previous explanation still holds.

SUMMARY

1. A case is reported, apparently unique, in which two symmetrical masses of slightly hyperplastic bone marrow were found attached to inner surface of the ribs in an old female hemiplegic, dying of terminal bronchopneumonia.

2. Various theories as to their etiology are considered. The autochthonous origin from heterotopic bone marrow cells or multipotent connective tissue cells, during embryonic life is thought more probable, but the possibility of their being benign bone marrow tumors must be considered.

3. Examples of myeloid metaplasia and bone marrow heterotopia from the literature are also considered, as well as the statement that

bone marrow structure is always found around the vessels in the hilum of the kidney and in the cortex of the adrenal in embryonic life.

4. A comparison is made with somewhat similar tissue, found in a case of osteitis fibrosa cystica.

The author wishes to express his appreciation to Dr. Krumbhaar for his help in preparing this paper.

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DESCRIPTION OF PLATES XI–XII

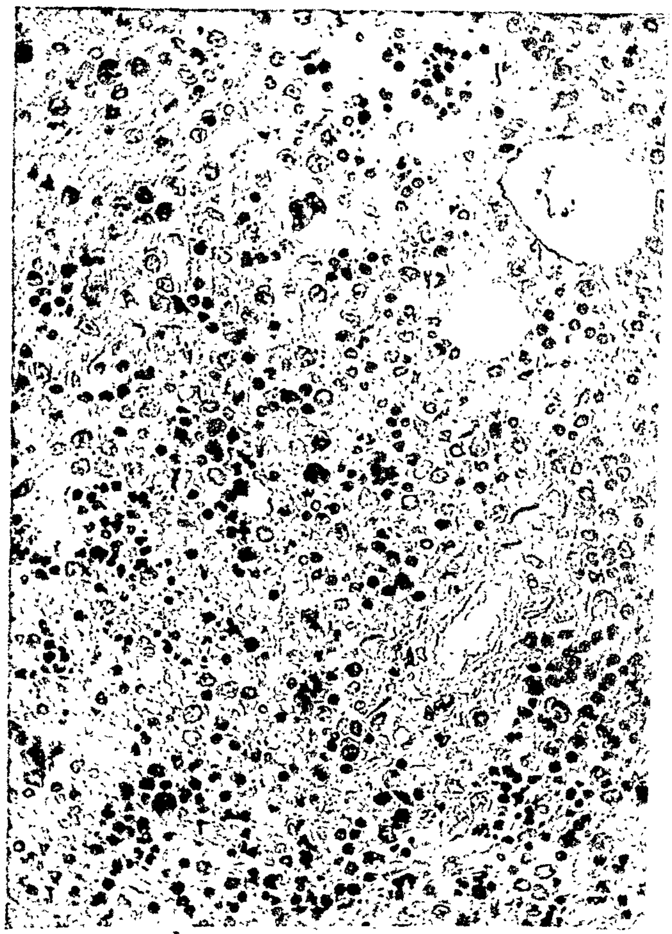
Fig. 1. Heterotopic bone marrow mass, external and cut surfaces.

Fig. 2. Histology of mass–hyperplastic bone marrow showing primordial cells; myelocytes, eosinophilic myelocytes, polymorphonuclear leukocytes, normoblasts, erythrocytes, megakaryocyte, fat cells. x 230.

Fig. 3. High power view showing leucogenetic and erythrogenetic centers. x 736.

Fig. 4. Margin of mass, showing macrophages, loaded with hemosiderin pigment. x 184.

Fig. 5. Goodpasture stain, showing oxydase granules in many of the cells. x 920.



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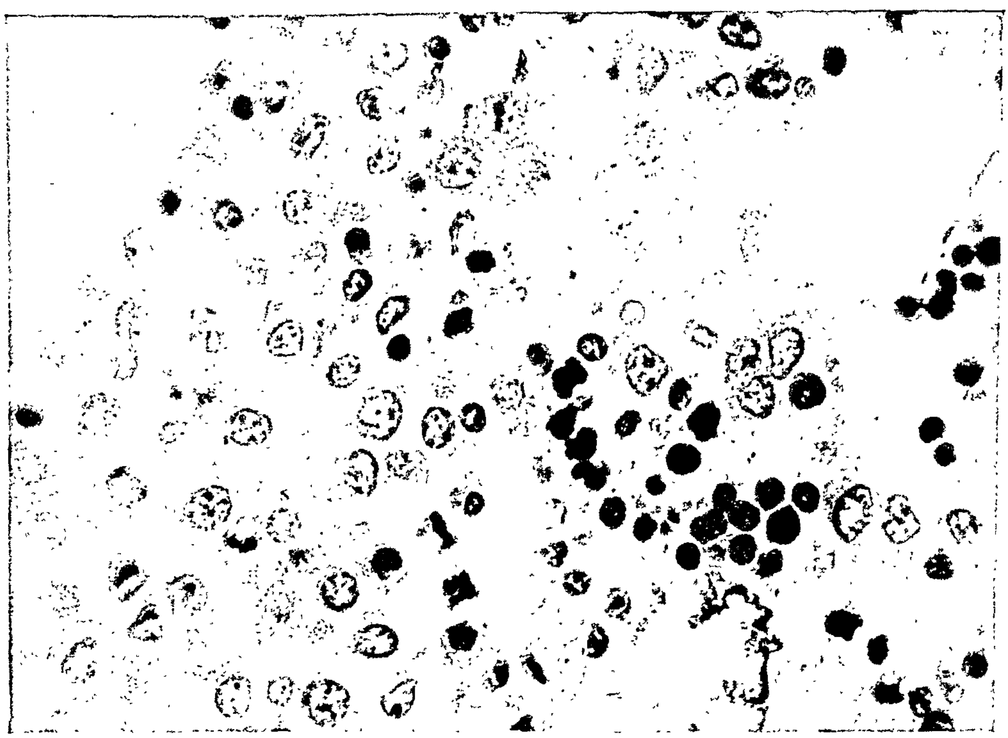

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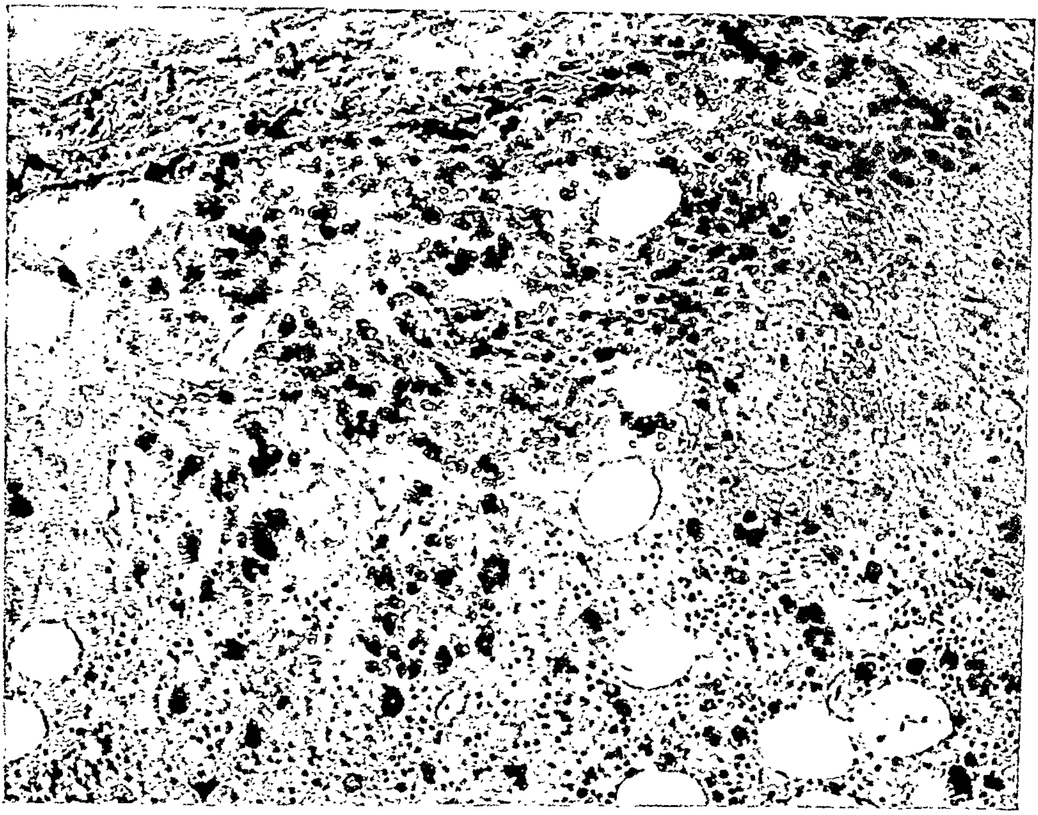
Saleeby

Heterotopia of the bone marrow



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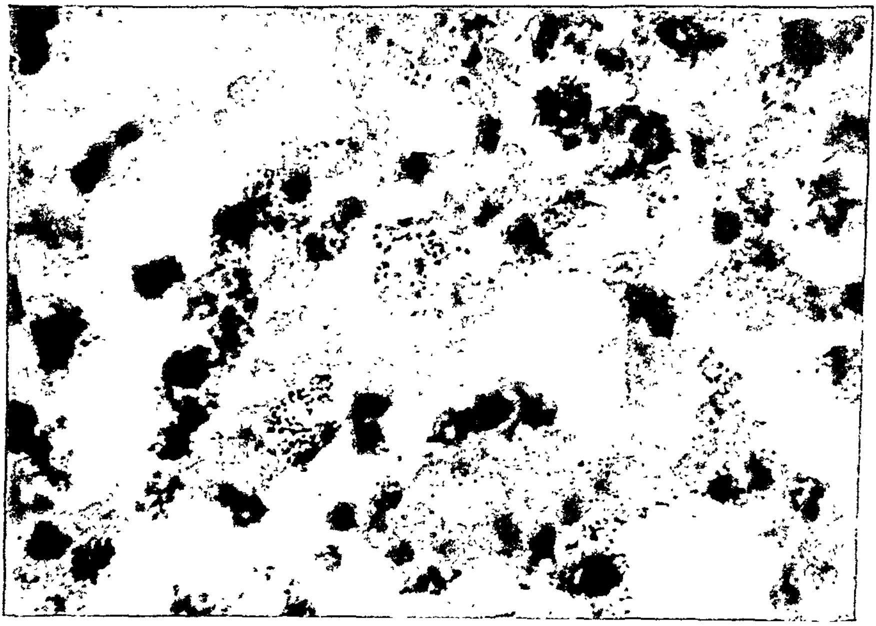

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Saleeby Heterotopia of the bone marrow

MICROGLIA AND THE PROCESS OF PHAGOCYTOSIS IN GLIOMAS*

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Recognition of a cell group in the central nervous system, distinct from neuron, neuroglia, and connective tissue, is an event of importance. Because of the nature of its well defined function, the activity of *microglia* is of particular importance in pathological conditions. Some of the stages in the activity of these cells in such conditions have long been recognized and bear familiar names: "Nissl's Stäbchenzellen," "ameboid wandering cells," "Gitterzellen," "granulo-adipose cells," "scavenger cells," etc.

There is a large group of cells, without previously recognized expansions, which are scattered everywhere throughout the central nervous system, and which have been called "apolar," "indifferent" or "adentritic cells," "naked nuclei" or "satellite cells," and "third element of Cajal".

Del Rio-Hortega has demonstrated that these "naked nuclei" are in reality possessed of complicated cytoplasmic prolongations and further, that we are dealing not with one class of cells but with two distinctly different groups.[22] One group, mesoglia of Robertson,[25] is considered by Rio-Hortega to be of ectodermal origin. He therefore prefers the name "neuroglia of few expansions" or "*oligodendroglia*" [17, 18].† The second subdivision, described by Rio-Hortega, has been called by him *microglia*.[19, 20]

* Received for publication September 4, 1924.

Some of the microscopical work was done in the Department of Pathology, Presbyterian Hospital.

† There has been some confusion on this point. Robertson, in a brief note, described a group of cells stained selectively by his platinum method. Without reporting any inquiry into their histogenesis, he assumed them to be of mesodermal origin. Because of the structure of these cells, and the fact that they are very numerous and appear frequently in rows between the fibers of the white matter, there can be no doubt that they are identical with oligodendroglia. These cells are probably of ectodermal origin (as maintained by Rio-Hortega) but final judgment on this point must await further histogenetic study.

The mesodermal origin and the function of microglia cells, on the other hand, has been described at some length and will be discussed briefly in this paper. Cajal, in

We have studied this element in human material and that of cats, rabbits and mice. It is present in both the white and gray matter of the brain and spinal cord. The nuclei of these cells are typically small and usually elongated. When stained by Nissl's method, they are seen to contain heavy chromatin granules. The cytoplasm and expansions can be impregnated by silver carbonate. Under normal conditions, microglia cells present many long attenuated expansions, quite irregular in shape and giving rise to numerous smaller branches and spines (Fig. 1 A). The shape of these prolongations depends somewhat on the pattern of the surrounding structure, as the microglial processes insinuate themselves into the interstices of the neuron warp.

The development of microglia is of considerable interest and throws light on its pathological changes. The idea of immigration into the central nervous system of mesodermal cells is a very old one. Thus Boll [4] in 1874 described movable cells in embryos which he believed to be neuroglia of mesodermal origin. The presence, in the brains of new-born infants, of granular ameboid cells, sometimes containing fat, led Virchow [28] to attribute their presence to "encephalitis intersticialis neonatorum," as early as 1867. These cells were variously considered to be normal and having to do with myelinization or to indicate an abnormal inflammatory process.

The cells described by these earlier investigators (complete reference is not attempted here) correspond to newly formed microglia, as demonstrated by the method of Rio-Hortega. These cells are few in number in the embryo until about the time of birth when there is a very rapid increase. This increase continues for the first few weeks following birth, after which time there is little change in normal mammals.

There are two chief "fountains" of microglia or places where the cells are first seen in large numbers, i.e., (i) the tissue beneath the ventricular ependyma at the site of the invagination of the pia to form the tela choroidea, (ii) the under surface of the cerebral peduncles. In these areas microglia cells are seen in large numbers immediately beneath the pia. From here there is a rapid spread of the cells along the clefts of the white matter. Thus, in the early days

1920, although accepting the establishment of microglia as an entity, was unable to satisfy himself that the rest of the adentritic or third element cells could be made to correspond to Rio-Hortega's description of oligodendroglia.

after birth, all of the microglia is in the white matter and none is to be seen in the grey matter, while after the first or second month the number in a field of grey matter is usually larger than in an equal field of white matter. The cytoplasm of the new-formed cells is finely reticular and contains small clear vesicles, or later vacuoles. During the stage of what appears to be migration, they take on various ameboid shapes with the frequent formation of pseudopodia. The transition from these migration forms to the fully ramified adult form can be seen with great clarity in brains (e.g., of rabbits) from two to ten days of age.

The author has never been able to see the actual transition from pial fibroblast to microglia cell as the method seems to stain cells only when they take on the aspect of microglia. Probably it is only when the mesodermal cells, which lie next to the brain, begin to ingest cerebral substances that they are rendered stainable by silver carbonate. Consequently, a layer of flattened microglia cells is found between the under surface of the pia and the nervous tissue. These flattened cells are not interspersed with larger ones engorged by phagocytosis. Such cells lying about blood vessels will be described below. The *possibility* that microglia may be derived as well from the adventitia of the larger cerebral vessels, about which they congregate, must be considered; also, the possibility of derivation from embryonal or polyblastic cells, whose existence has been urged by many investigators, cannot be denied.

In pathological conditions involving a destruction of the cerebral tissue, e.g., about areas of softening, there is a rapid and easily demonstrable reversion of the microglia in the neighborhood to ameboid forms (Rio-Hortega,[20, 21, 23]). That is, the complex expansions become less ramified and the cytoplasm approaches its nucleus. The cell migrates to the site of the lesion and takes on the well known characteristics of Gitterzellen (Abräumzellen, granulo-adipose cells). Collado[8] has described somewhat similar changes of microglia in cases of rabies.

Metz and Spatz, in a recent paper,[13] confirm the morphological character of microglia ("Hortegaschen Zellen") described above and the conversion of this element into "Gitterzellen." But they believe these cells to be fixed and non-migratory. They continue to agree with the German investigators who preceded them that Gitterzellen may likewise be formed by neuroglia and fibroblastic cells. If the

phagocytes formed by these last two types of cell possess the power of movement, as they admit, it is difficult to understand how the microglia, in its transformation to Gitterzellen, can remain a fixed element in pathological conditions. They incline to the view that microglia is not of mesodermal origin but is a type of neuroglia. Nevertheless, there is in their most interesting paper a new proof of the functional differentiation of these two types of cell; i.e., that in general paralysis iron pigment is found in large amounts in microglia while none is to be seen in neuroglia cells (nor, for that matter, in nerve cells).

In experimental brain wounds of short duration, studied by the author, the change in the form of microglia cells is so well graduated at different distances from the lesion as to leave no doubt that microglia develops *phagocytic* and also *migratory* power, thus surrounding the lesion in a very short time with a cluster of granulo-adipose cells (see also Rio-Hortega[21, 23]). These early sections contain no evidence of a similar metamorphosis on the part of other types of cells. It is, of course, impossible on these grounds to maintain that granulo-adipose cells can never be derived from other types of cells under different conditions.

Finally, it must be admitted that the actual transition stage from fibroblast to microglia has not yet been demonstrated. Nevertheless, because of the nature of microglia reaction to pathological conditions, its resemblance to macrophages seen in other parts of the body, the absence of any transition between it and neuroglia, and, most of all, because of the location and time of appearance of the "fountains of microglia" as well as its manner of invasion into the central nervous system, microglia must be considered of mesodermal origin. It forms, therefore, a third element in the central nervous system.

Case I* is that of a patient (A.R.) in the Presbyterian Hospital, New York City. Briefly, the history was of more than a year's duration, the chief characteristics being gradual mental change with headaches and several attacks of unconsciousness. At operation, the author turned down a large bone-flap exposing an extremely tense dura. A needle, passed through the dura into the left frontal lobe, yielded a small amount of clear yellow fluid. The bone-flap was

* The presence of microglia in a number of gliomas not reported here was established, although the conditions prevented perfect staining results.

therefore closed at once with a tentative diagnosis of cystic glioma. The patient died six days later and autopsy showed a large infiltrating tumor which involved both frontal lobes and contained two small cysts, one of which was filled with blood (Fig. 2).

Over a year later blocks were taken from the tumor and stained by Rio-Hortega's silver carbonate method for microglia.* As judged by these sections and the routine sections made at the time of autopsy, the tumor is a glioma. It contains a moderate number of nerve cells (cf. 27, 16). The neoplastic cells grow close together in some areas and in a lax formation in others. Occasionally they are arranged in small rings, calling to mind the structure of the ependyma. (Stroebe,[27] Mallory,[12] Bonome,[5] Ranke,[16] Spiller, Bailey.[2])

Microglia in various stages of migratory and phagocytic activity can be seen everywhere throughout the tumor. By the silver carbonate method these cells and the fibers and cytoplasm of giant neuroglia cells are stained selectively, while only the nuclear outlines of the neoplastic cells are visible. With this method it will frequently be found that only the pathological neuroglia is stained.

These giant cells (Fig. 3) contain large irregular nuclei which are often multiple or in process of amitotic division (cf. Achucarro [1]). In some cases daughter nuclei appear to be leaving the cytoplasm (Fig. 3, A and B, see also Borst [6]). These cells elaborate long well-formed fibers which take the silver stain energetically (Fig. 4) and pass out a considerable distance through the neoplastic parenchyma, providing the greater part of the fibers visible in the tumor. Stroebe, in 1895, and Ranke, in 1911, among others, suggested that most of the fibers seen in gliomas are produced by previously existing neuroglia as a reaction to the presence of the tumor. The cytoplasm of these cells is large in amount and at times contains vacuoles shown by Mallory's stain (Fig. 3), or granules when impregnated with silver (Figs. 5 and 6). This change in cytoplasm indicates degeneration and is accompanied by fragmentation and disappearance of the prolongations and by gradual loss of the cell outlines (Fig. 7).

Such regressive change in neuroglia cells was taken by Rosental [26] to be preliminary to an ameboid wandering stage, a transformation

* The author did not succeed in interpreting these sections until he had later studied the development and behavior of microglia under the generous personal guidance of Dr. del Rio-Hortega, who also called to his attention the fact that no study has been previously reported of the relation of microglia to gliomas.

he reported in various pathological conditions of the brain. His illustrations show clearly first, degeneration of neuroglia cells similar to that described above, and second, smaller cells said to be ameboid glia but which resemble ameboid forms of microglia. Held's migratory cells, described in normal brains, may have been of a similar nature. Likewise, Alzheimer believed in a regressive transition from neuroglia to "Gitterzellen." Bonome [5] was of the opinion that the granulo-adipose cells found in gliomas were largely of fibroblastic origin, arising from the vascular adventitia, but he also believed a small proportion of these macrophages were derived from the regression of giant neuroglia cells.

In an excellent paper written in 1914, Ziveri [29] maintained that the above change in neuroglia was analogous to degeneration of nerve cells and that the cellular changes were of similar character. Although he had not at his command methods for staining the intermediary stages, he urged that it was the third element of Cajal which had to do with the catabolic changes in the central nervous system and not neuroglia. With a modification of Alzheimer's method, he described giant neuroglia with fragmenting expansions and about these cells grandulo-adipose cells. Unfortunately, no conclusions can be drawn from his illustrations, but he is of the opinion that the function of the third element is *similar* to that of granulo-adipose cells.

Whether or not the giant neuroglia cells in the tumor under consideration arose from the tumor itself or were originally proper to the brain structure is only a matter of conjecture. Certain it is that these fibrous neuroglia cells resemble at certain stages the giant glia seen in other pathological conditions of the brain.

There is no evidence that these degenerating forms take upon themselves phagocytic activity.* The changes are *purely degenerative* in character. With fragmentation of the fibers (Fig. 6), the cytoplasm becomes filled with argentophile granules and vacuoles which may eventually be represented only by fine dust (Fig. 7). The cell outlines become progressively fainter and eventually indistinguishable. The cause of this degeneration is not apparent. It

* Cajal (Trab. d. Lab. Invest. Biol. XI, '13, bottom of p. 297) noted hyperplasia of the neuroglia about experimental brain wounds and the evidence of phagocytosis on the part of third element cells but found no indication of transition from one type of cell to the other.

does not seem to be due directly to lack of blood supply in the neighborhood of the cells themselves, for such changes are seen in cells which are close enough to functioning vessels to implant vascular feet on their walls (Penfield [14]).

The rôle played by microglia in this process of neuroglia degeneration is of considerable interest. In early stages microglia cells are found in relation with the *neuroglial processes*, showing a predilection for smaller processes. Each cell applies itself to a fiber or engulfs its termination in greedy cytoplasm (Fig. 5, A, B, C). Later, as the prolongations begin to break off, the macrophages approach more closely and envelop fragments (Fig. 6, A, B, C), or absorb the stumps of large prolongations (D, E, F). There comes a time, however, when the stumps of the dismantled cell no longer attract microglia (Fig. 6, G, and Fig. 7). At no time was phagocytosis of the cell body seen.

During digestion of the fiber fragments, microglia cells appear to pass through several characteristic phases. When a cell first becomes applied to a fragment it usually contains a somewhat oval nucleus at the end of an elongated bag-shaped protoplasm. The nucleus is unstained and the cytoplasm faintly impregnated and possessed of very fine scattered granules, most noticeable at the cell surface. Argentophile granules appear about the nucleus, especially at one pole (Fig. 8, A). The cytoplasm of these cells may take any shape, however, and the uniformity of outline is due to the constant shape of the subjects of phagocytosis. Where more than one fiber is being engulfed as B, C, D (in Fig. 8), this form varies according to the situation. The argentophile granules become larger first near the nucleus and later in the rest of the cell body (Fig. 8, E). These granules, or, better, globules, become numerous and the cell assumes a more or less spherical form (Fig. 8, F, G). Such forms are usually found near vessels. So much is this the case that one is forced to assume migration of the cell at this stage to the vessel. Cells H and K with unstained large vacuoles, no doubt represent some phase in fiber digestion.

In some regions microglia, scattered through the neoplasm, seem to be almost exclusively devoted to phagocytosis of neuroglia processes. There are other areas where the microglia cells, for the most part spherical, crowd closely and are filled with colorless vacuoles. These typical "Gitterzellen" indicate destruction of cerebral tissue.

Often the intercellular spaces in such areas contain argentophile droplets and these the macrophages ingest (Fig. 9, A, B, C).

Certain areas, which contain no evident products for phagocytosis, present many microglia cells whose ameboid shape suggests migration (tuberous and pseudopodic forms of Rio-Hortega) (Fig. 9, E). These shapes tend to be parallel as though the direction of the migration were common to all.

There are likewise areas of microglia proliferation where mitotic figures are plentiful. The tissue of these areas is sufficiently lax to allow the cells to take on rounded forms and many free argentophile droplets are present. The various forms drawn in Fig. 10 were all found within a small compass. Some cell outlines were very indistinct, others easily visualized. No division by amitosis was encountered.

Examination of the blood vessels gives ground for certain interesting suppositions concerning the activity of microglia cells. In the vicinity of vessels is to be seen a large proportion of cells heavily laden with granules. Only the refractile outline of the vascular wall can be seen. Microglia cells are found plastered over the outer surface of vessels in the tumor. Various stages in the reduction of cytoplasmic contents and size can be seen in Fig. 11. The appearance of cells A and B suggests new arrivals heavily laden, C to G, stages in delivery of the cytoplasmic contents, and M and N, cells almost ready for renewed phagocytosis.

Such close relationship of granulo-adipose cells to the vessel wall has been taken by many investigators to mean that these cells arise from *adventitial* fibroblasts. The possibility of such origin in some conditions cannot be categorically denied, but Rio-Hortega's specific method enables one to see all stages of these cells with a clarity denied to previous observers. The constant phases illustrated in Fig. 11 suggest a delivery of cell contents, perhaps by osmosis, through the cell wall into the vessel (or perhaps into the perivascular space). If this is the case, one microglia cell may be many times liberated for its proper activity as a scavenger.

Forms O and P (Fig. 11) were seen in the relation illustrated, about various vessels. They are microglia without cytoplasmic inclusions and therefore ready for phagocytosis. They resemble closely cells in the first stage of phagocytosis of fiber fragments described

above (Fig. 8 A). The transition from heavily laden cells to these smaller forms is uninterrupted and without evidence of new formation. The conclusion, in this case at least, is that microglia cells migrate to the vessels, deliver their cytoplasmic contents through the vessel walls or into the perivascular space and return to a *renewed phagocytic activity*. Contributory evidence toward the truth of such a supposition is provided by the active mitotic increase in microglia cells which was seen to be independent of blood vessels (Fig. 10). It is also of interest to point out just here that Metz and Spatz [13] have shown that in general paralysis iron pigment is found exclusively in microglia *and* cells of the vessel wall.

Case II is that of a child of 8 years in the Hospital Clinico de la Facultad de Medicina, Madrid. From the age of seven she had complained of failing vision, followed by blindness, convergent squint, headaches and vomiting. Shortly after admission to the hospital the patient died and autopsy showed a tumor of the roof of the midbrain, extreme hydrocephalus and "enormous swelling of the optic nerves."

Blocks of the tumor were sent by Prof. Suner to Dr. del Rio-Hortega, to both of whom I am indebted for the opportunity to study the material. The neoplasm is a glioma. Its cells appear fibrous in places and are closely packed. In other areas the structure is more lax. Ependymal ring formation is to be seen occasionally. Fibers of neuroglia type are found to be plentiful when stained by the silver carbonate method for neuroglia. There are areas of marked softening in the tumor.

When stained by Rio-Hortega's method for microglia, these cells (microglia) are found to be plentiful in zones encircling the foci of tumor softening. Figure 12 shows such an area of degeneration (A). Vessels in this focus and at its border are thrombosed (H). The zone between neoplasm (N) and softening (S) is occupied by microglia cells in active *dendrophagocytosis*. The neuroglia fibers in the zone are evidently degenerating as they take the stain intensely and are being rapidly devoured. No microglia cells were found in well vascularized areas of tumor nor within the focus of softening.

In this case, as in the first case reported, the activity of microglia within the neoplasm is restricted to dendrophagocytosis. It seems therefore that microglia cells subserve two principal functions in re-

lation to gliomas, i.e., phagocytosis of degenerating neuroglia fibers within the tumor and phagocytosis of the products of cerebral destruction in the brain surrounding the neoplasm.

Large phagocytic cells have been recognized in tumors of various types in other tissues. They have been considered as derivatives of one or more of the following cell forms: fibroblasts, vascular endothelial cells, leucocytes, and ubiquitous undifferentiated polyblastic cells. In a recent publication,[24] Rio-Hortega and Asua have described fully the structure of these cells. They call attention to the striking morphological similarity between the above macrophages and the phagocytic cells of the central nervous system (ameboïd forms of microglia).

Conclusion

The activity of microglia in the brain surrounding a glioma is similar to that described by Rio-Hortega in other destructive processes of the central nervous system. These microglia cells act as scavengers to clear away the products of degeneration and cerebral destruction.

Giant neuroglia cells in the neoplasm undergo degeneration with fragmentation and loss of their processes. This change is *purely degenerative* and is not a stage in the formation of ameboid phagocytes. The supposition that such a change was possible has probably been due to confusion of this degenerated cell with phagocytic microglia. That neuroglia cells ever metamorphose into microglia appears to be extremely improbable.

In the nervous tissue surrounding the tumor microglia cells take on ameboid forms. The relation of these cells to the degenerating neuroglia within the neoplasm is a constant one, consisting of phagocytosis of the prolongations (dendrophagocytosis) but not of the cell body. In areas of cerebral softening the microglia cells crowd closely, assuming the more spherical and reticulated form typical of "Gitterzellen." They resemble the macrophages of fibroblastic origin seen in various neoplasms, as described by Rio-Hortega and Asua.

Regions of very active mitotic division are to be seen. This cellular reproduction does not involve the intervention of either neuroglial or adventitial cells.

Microglia cells seem to transport the ingested substances to the

outer surface of blood vessels where they lose their granular and vacuolar appearance, decrease in size, and finally leave the vicinity of the vessel in characteristic form for renewed phagocytosis. The inference is made that the digested contents of these cells pass into the vessel lumen or into the perivascular space. The presence ot scavenger cells about vessels indicates transfer and delivery of ingested substance rather than new formation of these cells from fibroblasts.

Microglia seems to discharge two chief functions with relation to a cerebral glioma: (*a*) within the tumor, dendrophagocytosis and (*b*) in the nervous tissue surrounding the tumor, phagocytosis of the products of cerebral destruction.

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DESCRIPTION OF PLATES XIII–XX

Fig. 1. Transition from normal microglia cell A to granulo-adipose cell E and F, characterized by shortening and swelling of expansions, swelling of cell body and vesiculation of cytoplasm. Cell A was taken from another case, as it is almost impossible to stain a normal cell completely after long fixation in formol, although pathological forms still stain. B to F are from brain adjacent to the tumor. All preparations illustrated were made by the Silver Carbonate Method of Rio-Hortega unless otherwise stated.

Fig. 2. Cross section of brain showing location and extent of tumor.

Fig. 3. Giant cells from tumor stained by Mallory's Phospho-tungstic Acid Hematoxylin Method. A, B, and C degenerating cells with vacuolar cytoplasm and nuclei which have undergone simple division; D, radiating partition of nucleus; M, nuclei of microglia; N, same with cytoplasm faintly stained.

Fig. 4. Two giant neuroglia cells. Microglia scattered among the faintly stained neoplastic nuclei.

Fig. 5. Giant neuroglia cell in early degeneration. The cytoplasm is granular and microglia cells A, B, C have applied themselves to its expansions at their terminations.

Fig. 6. Giant neuroglia cell in advanced degeneration. A, B, and C, microglia ingesting fiber fragments. D, E, and F, microglia applied to stumps of the remaining expansions. Expansion G, as well as the cell body, apparently does not attract phagocytes.

Fig. 7. Final stage in degeneration of neuroglial cells.

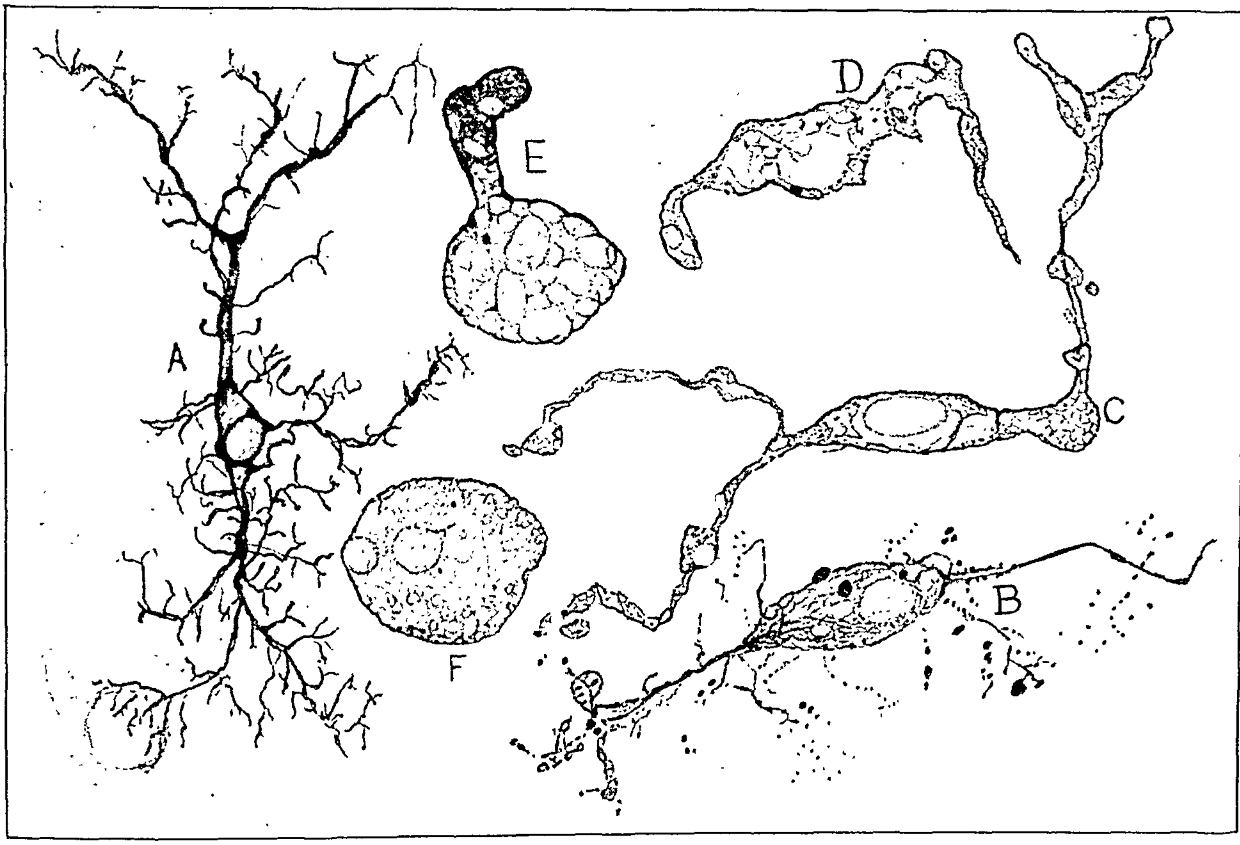
Fig. 8. Stages of ingestion of glial fiber fragments by microglia, illustrating the manner in which the cytoplasm follows the fiber form. A, earliest stage, to G, final stage. H and K are stages in fiber digestion.

Fig. 9. Ameboid phagocytic microglia ("Gitterzellen," "granulo-adipose cells").

Fig. 10. Mitotic division of microglia.

Fig. 11. Blood vessel in glioma surrounded by microglia in various stages of delivery of ingested substance (A to G). M to P, emptied cells.

Fig. 12. Area of softening (S) within glioma, case II, surrounded by a zone of microglia in active dendrophagocytosis (B). H, thrombosed vessels. N, neoplasm.



Penfield Microglia

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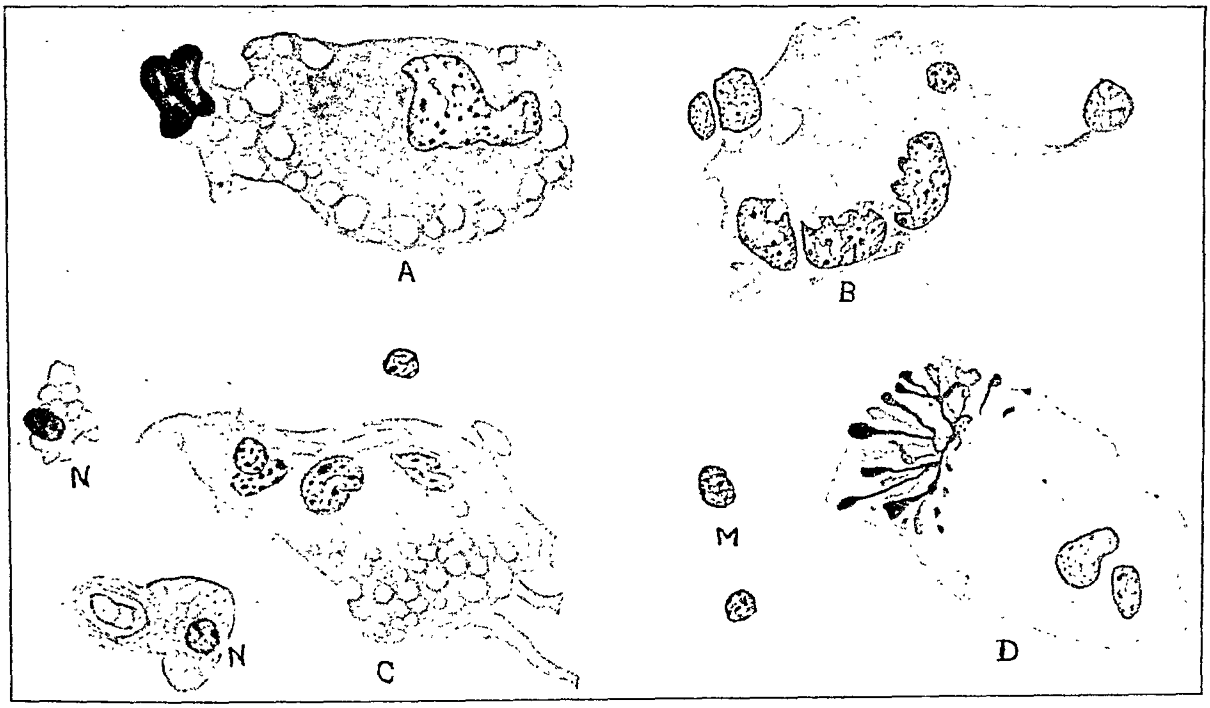

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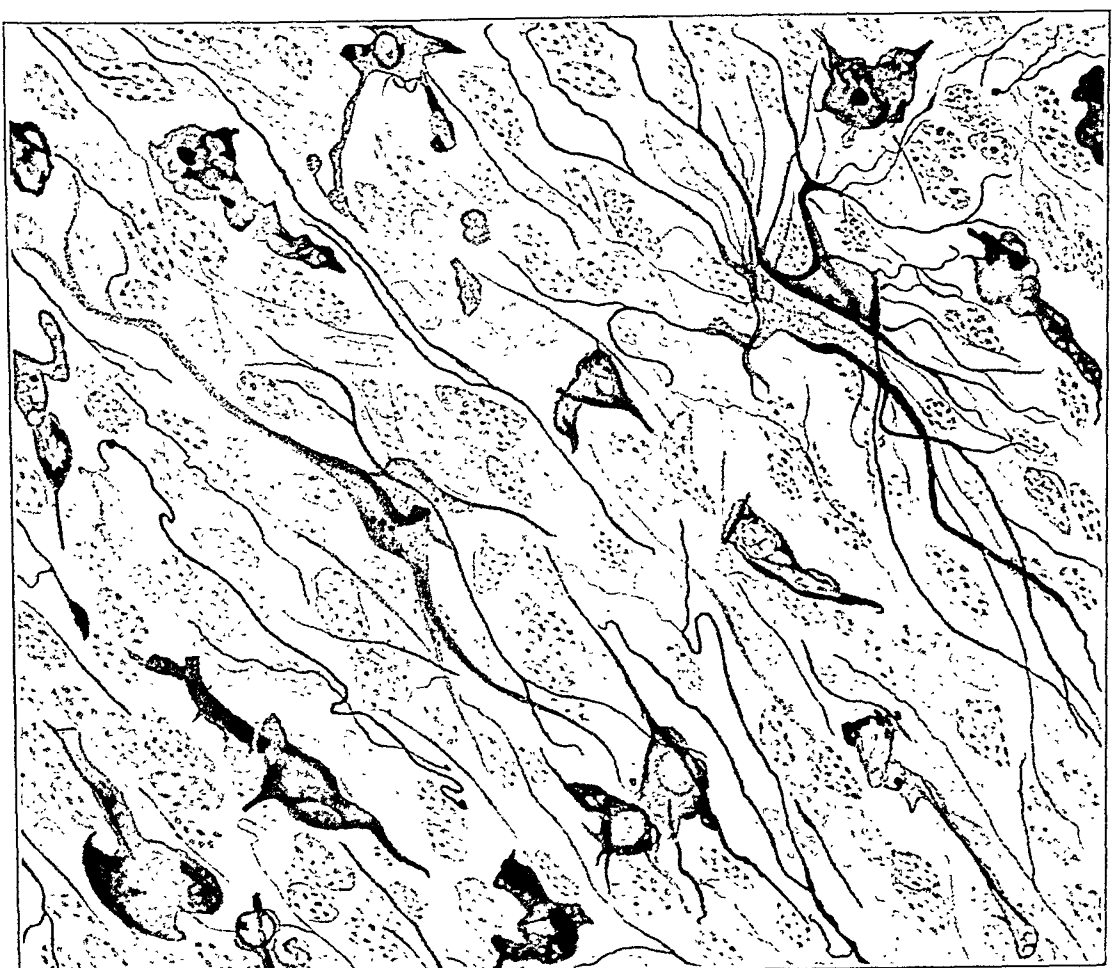

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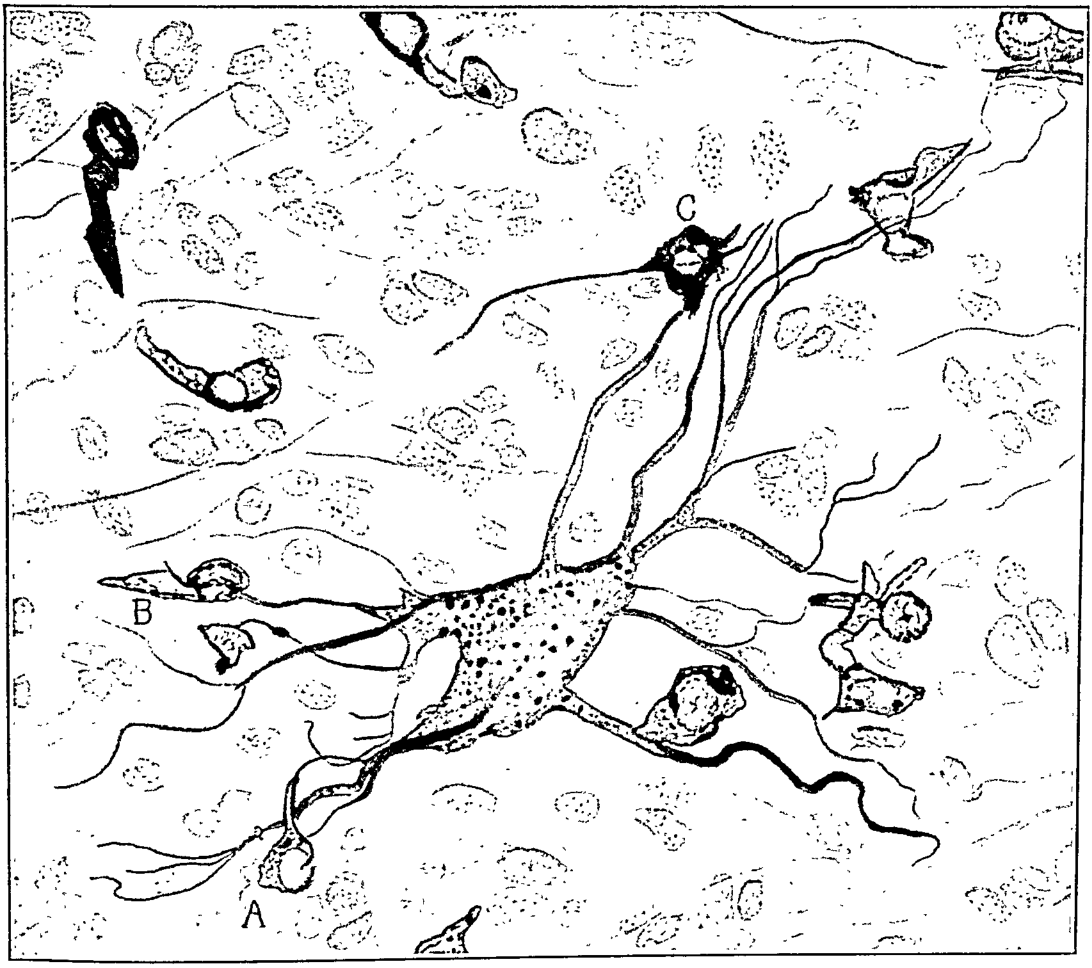
Penfield

Microglia



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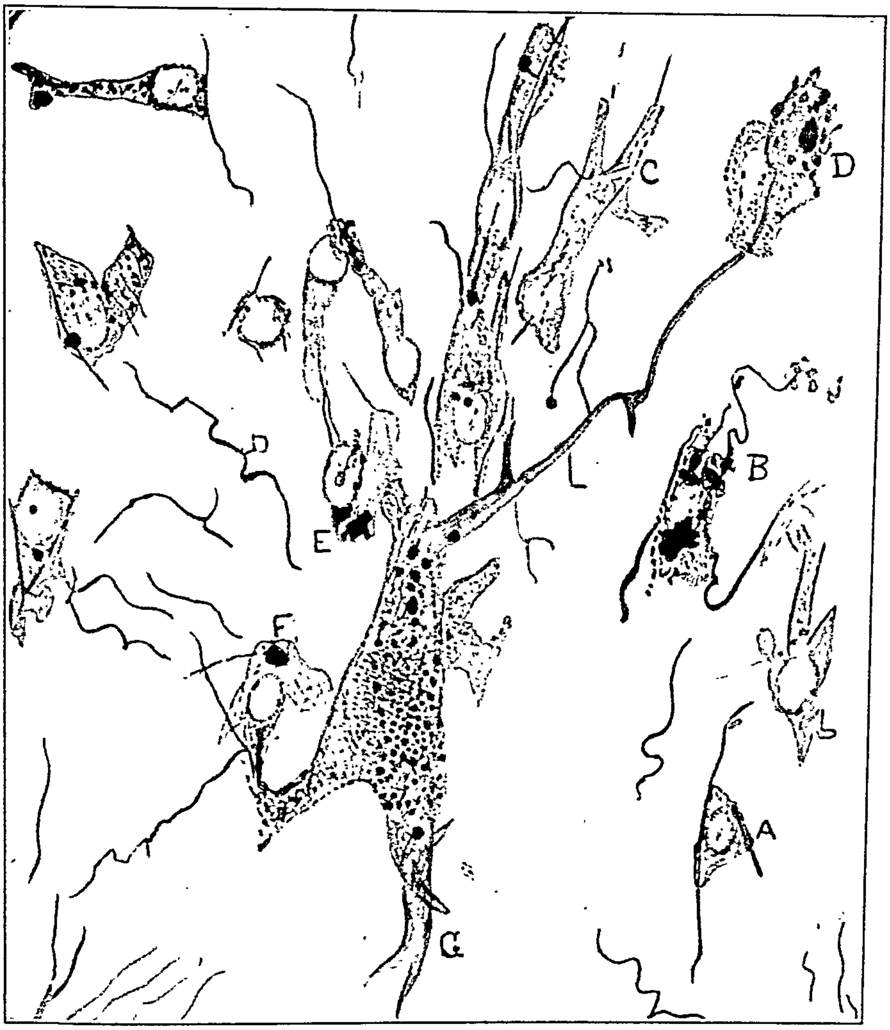

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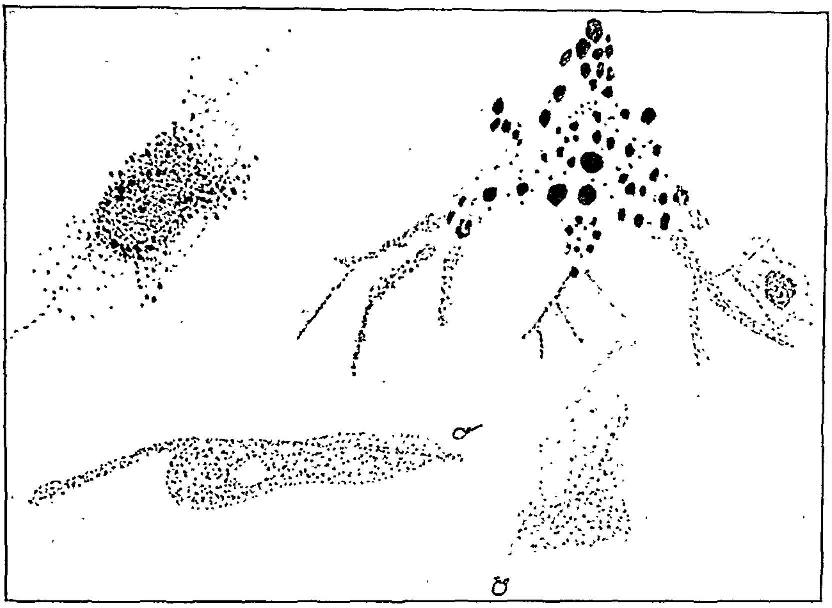

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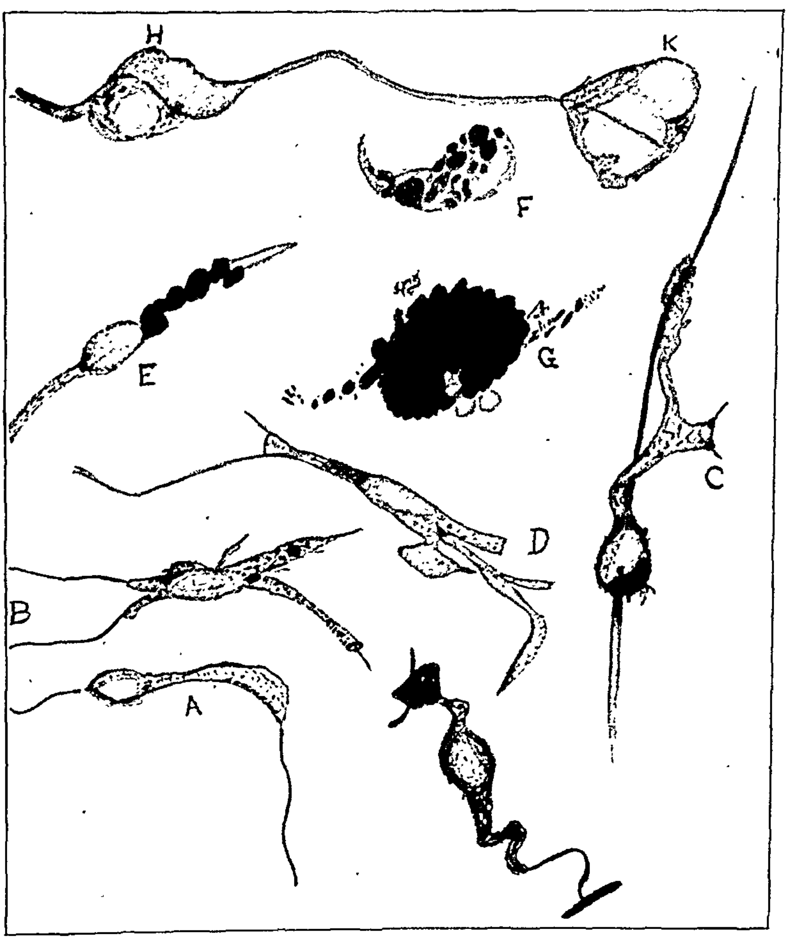

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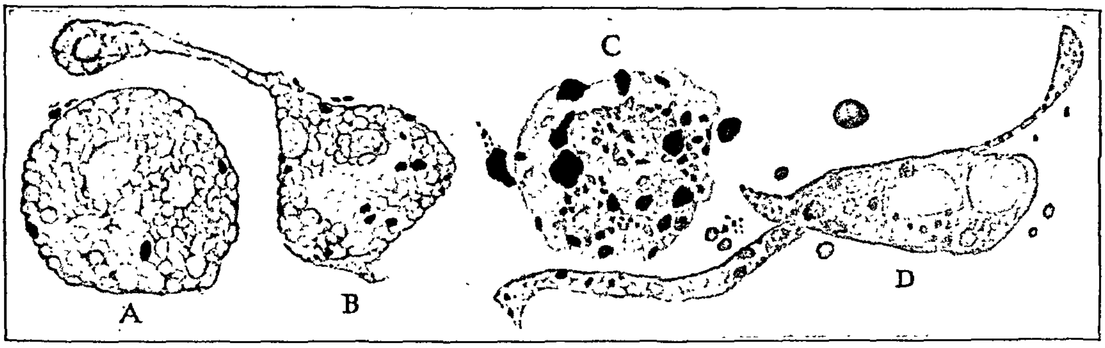

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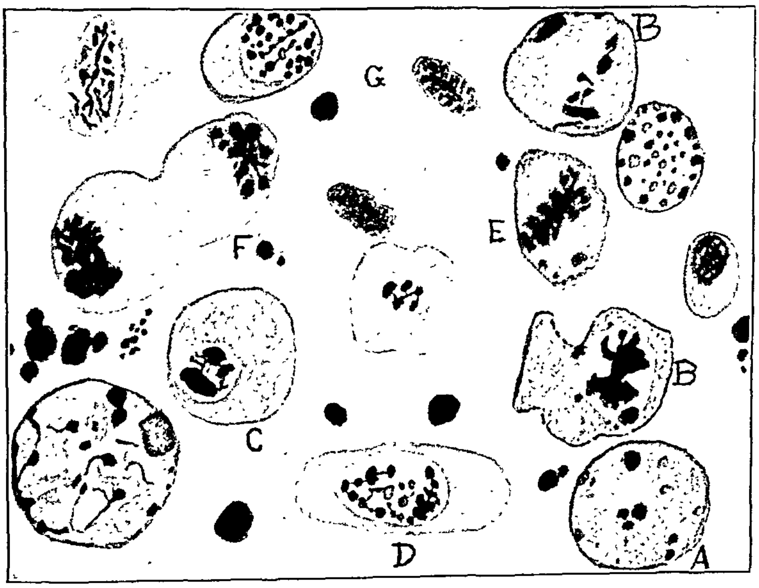

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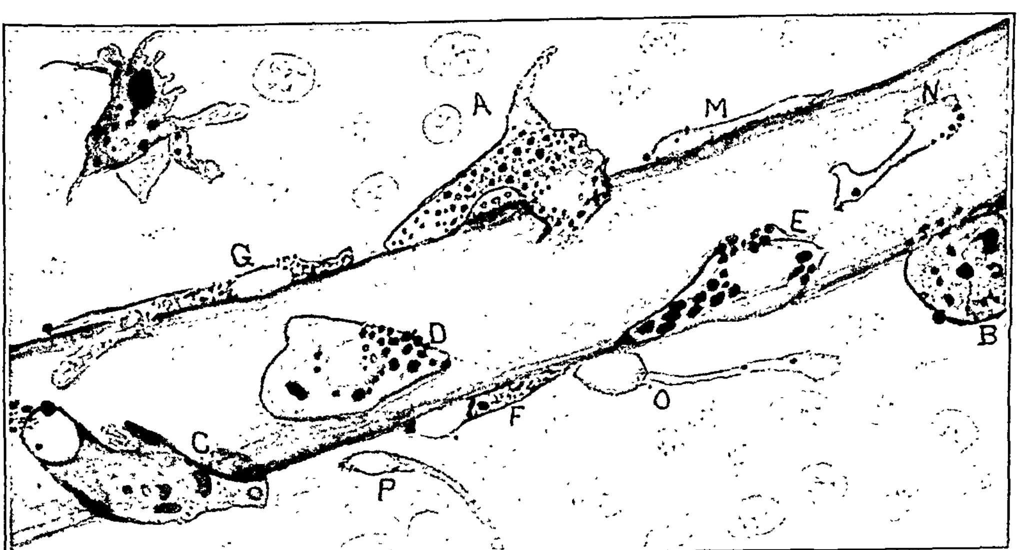

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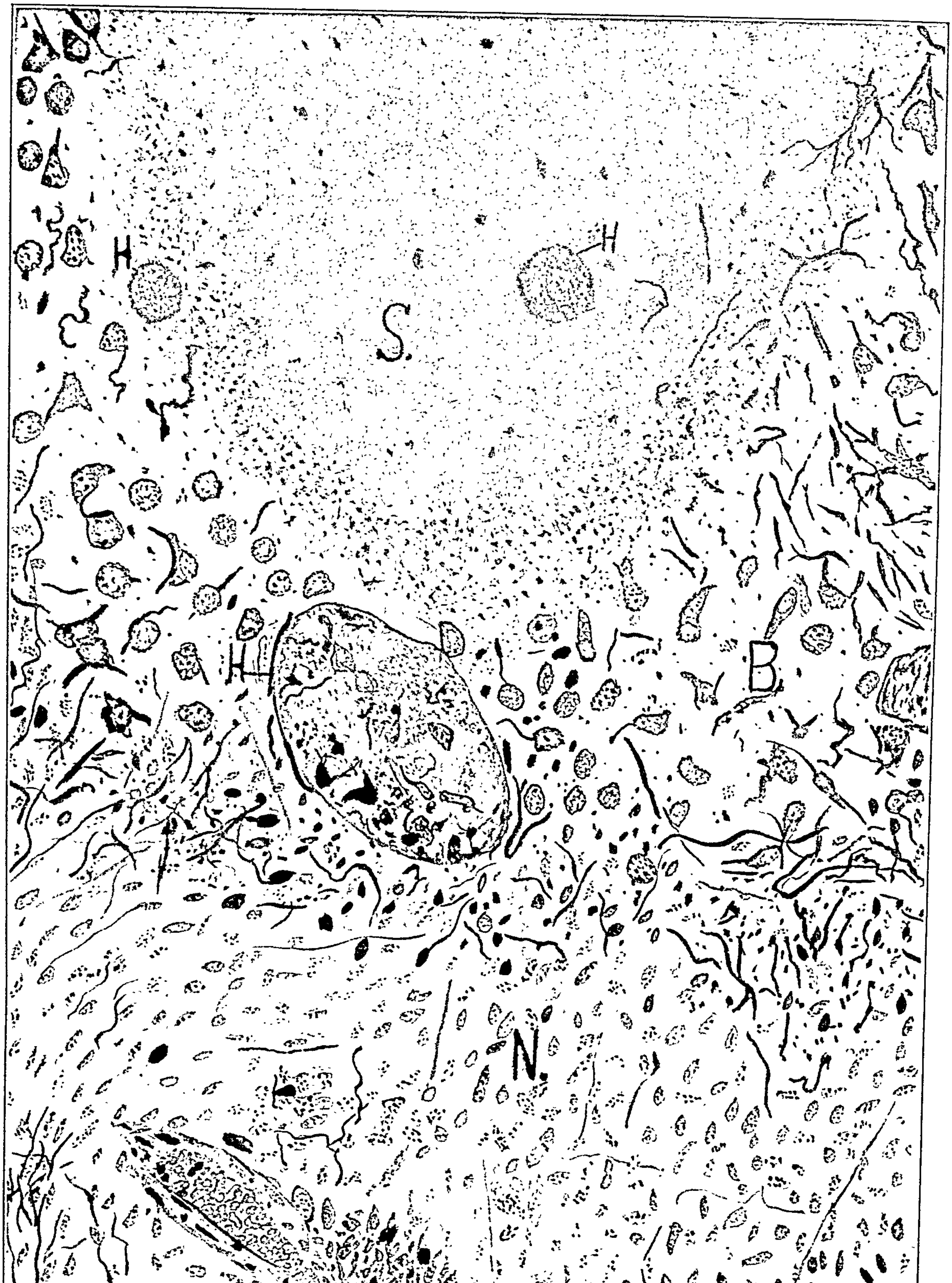

12

THE FORMATION OF MACROPHAGES, EPITHELIOID CELLS AND GIANT CELLS FROM LEUCOCYTES IN INCUBATED BLOOD *

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While tissue cultures would seem to afford an appropriate technic for the study of the part taken by the white blood-cells in various conditions, this method has seldom been employed. In 1914 Awrorow and Timofejewskij studied the white blood-cells of leukemic blood in plasma cultures, Loeb (1920) followed the amebocytes of the king crab in clotted blood, and Carrel (1921) observed the growth of the buffy coat of the centrifuged blood of the adult chicken in plasma cultures. The method by which the observations incorporated in this paper were made is simpler than that of any of the above investigators, consisting merely of the incubation of hanging drops of blood taken, by means of a paraffined pipette, either from the heart or from the peripheral circulation.

The transformation and growth of the leucocytes into macrophages, epithelioid cells, and giant cells were observed in the blood of the chick embryo, young chicken, adult hen, mouse, guinea-pig and dog, and in human blood. In every kind of blood examined there developed first a large wandering cell, several times larger than any of the normal leucocytes, which was phagocytic for red blood-cells, melanin granules, carbon particles, dead granulocytes, and tubercle bacilli. Somewhat later there appeared a cell more like a primitive mesenchyme cell, and still later the epithelioid cell was formed. This cell was sometimes binucleate and in some instances a typical multinucleated giant cell (Langhans giant cell) was formed. Since the transformation and growth of the leucocytes were much the same in the different bloods examined, the details of the phenomenon in avian and human blood only will be described.

Avian Blood. The transformed cells occurred in incubated blood taken from the adult fowl as well as from embryos of various ages, the youngest used being 6 days' incubation. Usually the blood

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studied was taken from chicks just hatched or just about to hatch, but several series were from chicks one week after hatching, and two were from young chickens two months old.

When the blood culture was studied within an hour or so after preparation, it had a number of granulocytes with large, usually spindle-shaped granules, and some non-granular cells (Fig. 1) migrating about on the coverglass. At this time these two types of leucocytes showed the usual difference in size, and all of them contained typical leucocytic nuclei. Within a few hours one or two refractive globules appeared in some of the non-granular cells (Fig. 2), which were then considerably larger than the granular cells. After twelve hours there were always several cells much larger than normal. These hypertrophied cells contained no specific granules, but usually a few refractive globules (Fig. 3). They grew in size and also divided, increasing in number so rapidly that often after two or three days the culture contained hundreds of them (1200 were counted in one drop) and only a few granulocytes. As the cells hypertrophied there was a coincident change in the nucleus, which lost its compact character and became larger, paler, and showed definite nucleoli (Fig. 4). After 48 hours, while most of them were from three to four times as large as the granulocytes, not only in length and width but also in thickness, smaller ones were still present (Fig. 5), some no larger than the original leucocyte. The cells now contained a number of refractive globules of different sizes, some quite large, most of which stained with Sudan III, while some appeared to be plasma granules. Such globules are not peculiar to the transformed leucocytes, as fat globules and plasma granules accumulate in most tissue cells grown in drawn blood or plasma. The cultures favorable for observation were those in which the cells did not form many of these globules.

After 24 to 48 hours the transformed cells were very phagocytic and took up various kinds of foreign bodies, but they seemed especially phagocytic for the red blood-cells of their own blood and most of them contained one or more erythrocytes, some of which were partly digested (Fig. 6). As many as twenty such cells were seen in a single macrophage. The ingested blood-cell soon died, as was indicated in preparations stained with neutral red by the fact that when first taken in the red blood-cell did not take the stain, but soon afterwards the nucleus became red and later the whole erythrocyte

became slightly red. Fragments of red blood-cells were also ingested and these stained red. In many instances the ingested cell crumpled up; in others it became laked, so that when stained it appeared as a fluid vacuole containing a red body — the nucleus. In later stages of digestion the nucleus of the ingested cell remained for some time after the rest of the cell had disappeared (Fig. 7). The macrophages resembled the clasmatocytes of the chick connective tissue to such an extent that it was difficult to distinguish the two cells from each other when placed side by side. The transformed leucocyte was usually somewhat larger and contained larger fat globules, otherwise each cell had the eccentrically placed nucleus, the thick cytoplasm, and the delicate, sheet-like processes continually changing shape and position. When stained with neutral red they each contained many red bodies scattered irregularly throughout the cytoplasm.

Some cells, migrating on the coverglass, were separated by a layer of serum from the red blood-cells so that they ingested few or none of the latter. These cells spread out on the glass, sometimes long and slender and joined end to end, or large and flat with a centrosphere. While these cells seldom contained ingested material they were nevertheless phagocytic; if the drop was shaken so that the erythrocytes came in touch with them, ingestion often followed and, as was observed in a few instances, the cells drew in their cytoplasm, became loosened from the glass, formed processes and became wandering cells. The clasmatocyte-like cells also occasionally became attached to the coverglass where they spread out into large flat cells in which a centrosphere was sometimes evident.

After the blood had been incubated 2 or 3 days some of the large flat cells became transformed into "epithelioid cells" (Fig. 10). By this term is meant, not all cells that resemble an epithelial cell, for that would include practically all of the large flat cells, but a specific large cell containing, around the centrosphere, a peculiar large central area which stains as a finely granular red area when neutral red is placed upon the preparation. This cell has a large nucleus with definite nucleoli usually eccentrically placed at one side of the central area. Beyond the central area, or just in its outer edges, there may be seen particles of ingested material and usually, in tissue cultures, many small fat globules in this outer region. Beyond this, the cytoplasm is extended out into an exceedingly delicate

peripheral film, the outer limits of which are almost impossible to distinguish in the living preparation, but which often become curled and drawn in, especially upon fixation, giving the characteristic curled edge so often seen in permanent preparations. Lewis and Webster (1921) described this cell in cultures of the human lymph node; Maximow (1924) observed the same type of cell in cultures inoculated with tubercle bacilli, and I have frequently obtained an abundant growth of this type of cell in cultures of isolated tubercles. The epithelioid cell developed earlier in chick blood than in the mammalian blood. The central area was neither so large nor so marked as in that of human blood, but larger than the centrosphere and stained in the characteristic manner.

Some of the transformed leucocytes became multinucleated. When this occurred, coincident with becoming epithelioid cells or later, the resulting giant cells were of the Langhans type. They contained from two to ten nuclei and were usually larger than the same type of cell containing only one nucleus, but not always so. The giant cells sometimes contained ingested material (Fig. 9) and in a few instances were observed to ingest red blood-cells, even after they contained several nuclei, but usually these cells digested what foreign bodies they contained and did not ingest any more.

The transformed leucocytes of the chick seldom lived more than seven to ten days in the incubated drops of blood, but if removed to plasma drops they lived for three or four weeks. It was in such preparations especially that the wandering cells became indistinguishable from clasmatocytes of the connective tissue subjected to the same environment. When plasma cultures of transformed leucocytes were incubated with avian tubercle bacilli the cells ingested large numbers of the latter. They digested the bacilli and behaved in every way as did the clasmatocytes when exposed to these organisms (Smith, Willis, and Lewis, 1922). In one such culture, fixed and stained after 20 days' incubation, there were observed, side by side, an elongated fibroblast-like cell (Fig. 12), a round epithelioid cell with a large central area (Fig. 11), and a clasmatocyte-like cell (Fig. 8), each containing tubercle bacilli.

When trypan blue or pyrrol blue was introduced into the culture, the granulocytes did not form blue granules; but after 48 hours the hypertrophied cells contained many, depending upon how much they came in contact with the stain.

Mammalian Blood. It was a simple matter to obtain an abundant growth of the transformed leucocytes of the chick's blood and practically every drop incubated contained many of them; but it was more difficult to get the same results with incubated drops of mammalian blood, and for this reason the following method was sometimes used as a control. Ten cubic centimeters of blood were rapidly drawn into a paraffined tube by means of an oiled cannula and centrifuged for about five minutes, most of the plasma withdrawn and the tube incubated. The buffy coat became a thick, tough layer of cells, extending up into the plasma and down among the red blood-cells. In this layer, after two to four days' incubation, the transformed leucocytes were abundant; many of them contained ingested and partly digested red blood-cells and some were multinucleated giant cells. These cells remained alive in the tube a number of days and living wandering cells were found in the bottom of the tube (two inches under the surface) even after ten days' incubation.

In the incubated drops of the blood of the mouse the cells grew well, regardless of whether the blood was taken from the heart or from the peripheral circulation. They did not multiply so extensively as those from the chick, but became many times larger than the normal cells (compare figure 14 with figures 15, 16, 17). They were phagocytic, as is shown in figures 15 and 17. The nucleus became large and contained definite nucleoli. Most of them were macrophages; a few were of the epithelioid type, as shown in figure 16.

The hanging drops of human blood were made from blood taken from the finger. These were usually injured by contact with the glass, and while the cells often lived for two or three days and displayed the beginning of the transformation, they more frequently died before the formation of an epithelioid type of cell, unless the coverglass was coated with some substance more favorable for their development. The best results were obtained by coating the covers with celloidin. In some of these cultures of human blood the white blood-cells lived for twenty days, in a few they lived nearly four weeks.

The first change in these cells also was the formation of refractive globules, which occurred after twenty-four hours, but the cells hypertrophied much more slowly than did those of the chick, so that it was often as long as four or five days before many unusually large

cells were seen. These cells were phagocytic and contained many red blood-cells; they increased in number until the eighth or ninth day. Scattered refractive globules of different sizes were present within them (Fig. 21), but these were much smaller than the globules in the hypertrophied cells of the chick blood. The nucleus became more homogeneous with more definite nucleoli. About the seventh day a few large flat cells appeared migrating on the coverslip. They were triangular, round, or spindle-shaped, and while at first they sometimes showed the remains of one or two ingested red blood-cells (Fig. 22), they soon completely digested these and took in no more. This was probably due to the fact that they did not come in contact with any, for if the blood was stirred, or fresh blood added, they again ingested red blood-cells, often in great numbers (Fig. 23). Instead of a few scattered refractive globules of different sizes, they contained many very small ones (Fig. 24). After eight or nine days these cells had increased greatly in number and had the appearance of epithelioid cells, i.e., they were large and flat with a large pale nucleus containing definite nucleoli, a large central body surrounded by a layer of fat globules and debris, and a delicate peripheral film of cytoplasm. When the preparation was stained with neutral red the central body became stained red (Fig. 26). A few of these cells became multinucleated and formed typical giant cells, but this was by no means so frequent an occurrence as in the cells of avian blood.

From the ninth day on, the majority of the hypertrophied cells formed in the human blood were of this type, although there were always present a few migrating phagocytic cells, containing red blood-cells, and some long spindle-shaped cells (Fig. 25). They did not retain the same shape from day to day; a round cell was observed to change into a long, thin, spindle-shaped cell and then into a triangular cell. These changes took place gradually and the cells migrated very slowly. They continued to increase in number for several days, after which they increased slightly in size and died when between three and four weeks old.

Although the transformed cells of the human blood lived so much longer than those of the chick, they never multiplied to the extent that the latter did. In cultures of chick blood, after a few days of incubation there were hundreds of these cells, but the greatest number ever seen in a single drop of human blood was 360 in a culture that had been incubated ten days. Occasionally a cell hypertrophied

rapidly and even ingested red blood-cells before the nucleus had lost its leucocytic character.

In regard to the red blood-cells, it is impossible to state how long those of the mammalian blood can live; they often seemed to be in good condition for as long as six or eight days. Those of the chick, however, are nucleated and form vacuoles which stain with neutral red, so that it could be determined that many of these remained alive for as long as eight days, as was shown by the fact that the nucleus did not stain with neutral red, while the vacuoles did.

Discussion. Whether more than one type of blood-cell hypertrophies and gives rise to the transformed cell is difficult to state. Awrorow and Timofejewskij conclude that the lymphocyte is the stem-cell from which arises the enlarged mononuclear cell and from it develop the other types of transformed cells found in their plasma cultures; i.e., the wandering cell, spindle-shaped cell, phagocytic cell, giant cell, and the cell which these observers call the "Auslauferzelle." They hesitate to call the cells either clasmatocytes or fibroblasts and, although they have found cells resembling the clasmatocyte and others resembling the fibroblast, these authors decided that, on the whole, the majority of the cells are not entirely like either of these types of connective-tissue cells. Carrel states that the granulocytes disappeared from his cultures after a few passages, and since he failed to find the small mononuclear after the first week, he supposed that these cells either were transformed into the large mononuclear leucocyte or died out, while the large mononuclear cells proliferated, migrated and, under certain conditions, changed into cells resembling fibroblasts and into transition forms, half fibroblast and half ameboid cells. He also observed typical macrophages in his plasma cultures. Clark and Clark (1920, 1923) were able actually to follow the living cells throughout their activity in sterile inflammation in the tadpole's tail. These investigators state the polymorphonuclear leucocytes migrated out from the blood-vessels toward the site of inflammation, where they became stationary, with spherical nuclei, sent out processes, and came to resemble fibroblasts; and that as the inflammation subsided, these cells again became ameboid and wandered away. In my cultures of chick, mammalian, and human blood, granulocytes were not observed to become transformed, and in blood containing a great

many of them there did not occur a proportionately greater number of transformed cells, nor did the hypertrophied cells contain neutrophilic or eosinophilic granules. So far as could be determined by following the cells in the incubated drops of blood, it seemed to be the mononuclear type that gave rise to the three kinds of transformed cells, i.e., the macrophage, the epithelioid cell, and the giant cell.

Sabin (1921, 1923) and her co-workers, Cunningham and Doan (1924), have been carrying on a series of most interesting observations in which they differentiate the various types of blood-cells by means of vital dyes, and on this basis attempt to establish a grouping of the blood-cells more in accordance with what they consider to be their origin. By this method these investigators, in their later publications, distinguish the monocyte from the clasmatocyte and claim that, while both the monocyte and the clasmatocyte are phagocytic, there is nevertheless a distinct difference between the two types of cells. In view of this it is rather interesting that some of the transformed leucocytes certainly resemble clasmatocytes, whether the term be employed in its usual sense of tissue macrophage or in the more restricted meaning assigned to it by these later writers. These clasmatocyte-like cells may not be true clasmatocytes, but neither can they properly be termed monocytes, owing to their increased size, changed nucleus, accumulated fat globules, irregularly scattered ingested material and neutral-red bodies. Just what term should be used to designate these cells does not decrease the importance of the fact that macrophages, epithelioid cells and giant cells do arise from the leucocytes of the blood, without, in this case, any possibility of participation in the phenomenon by the endothelium or the connective tissue.

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DESCRIPTION OF PLATE XXI

Transformed leucocytes in the incubated drops of blood, drawn while living. Ocular No. 6, 2 mm. lens. [Reduced one-half in reproduction.]

Fig. 1. The normal cells of avian blood. These were drawn one hour after the cultures were prepared. Granulocyte, mononuclear, lymphocytes, and nucleated red blood-cell.

Fig. 2. Non-granular cell in blood from a 2-months' old chicken after 6 hours' incubation.

Fig. 3. Hypertrophied non-granular cell in blood from a 2-months' old chicken, incubated 12 hours.

Fig. 4. Transformed leucocyte containing fat globules and red blood-cells in same culture as that of Figs. 2 and 3, after 24 hours' incubation. The nucleus has lost its leucocytic character.

Fig. 5. Phagocytic cell containing a red blood-cell in same culture as that of Fig. 8. This cell had not hypertrophied.

Fig. 6. A phagocytic wandering cell containing many red blood-cells. From the blood of a week-old chicken, incubated 3 days.

Fig. 7. A phagocytic wandering cell spreading out on the coverglass. It contained laked red blood-cells and the nuclei of many digested ones. From the blood of a 2-months' old chick, incubated 4 days.

Fig. 8. A phagocytic wandering cell which was transferred to a drop of plasma containing avian tubercle bacilli. This cell ingested many organisms and lived for 20 days.

Fig. 9. A giant cell containing partially digested red blood-cells. Blood from a week-old chicken — after 4 days' incubation.

Fig. 10. An epithelioid cell in a drop of blood from a 19-day old chick embryo, after 3 days' incubation.

Fig. 11. An epithelioid cell containing many tubercle bacilli. From the same preparation as that of Fig. 8 — after 4 days in plasma.

Fig. 12. A long spindle-shaped cell containing ingested avian tubercle bacilli in same culture as that of Figs. 8 and 11.

Fig. 13. Red blood-cell from the blood of the mouse.

Fig. 14. Mononuclear cell from the blood of the mouse.

Fig. 15. Phagocytic cell from a drop of mouse blood incubated 40 hours.

Fig. 16. Round flat cell of the epithelioid-cell type from a drop of blood of the mouse, taken from the abdominal circulation and incubated 48 hours.

Fig. 17. Phagocytic cell from a drop of blood from the heart of the mouse, incubated 48 hours. It contained many red blood-cells, each of which was in a different stage of digestion.

Fig. 18. Mononuclear cell from human blood, one half hour after the culture was prepared.

Fig. 19. Polymorphonuclear cell from human blood, one half hour after the culture was prepared.

Fig. 20. Human red blood-cell, one half hour after the culture was prepared.

Fig. 21. Phagocytic wandering cell of the clasmatocyte type, containing ingested erythrocytes; drawn after 7 days' incubation of human blood on celloidin.

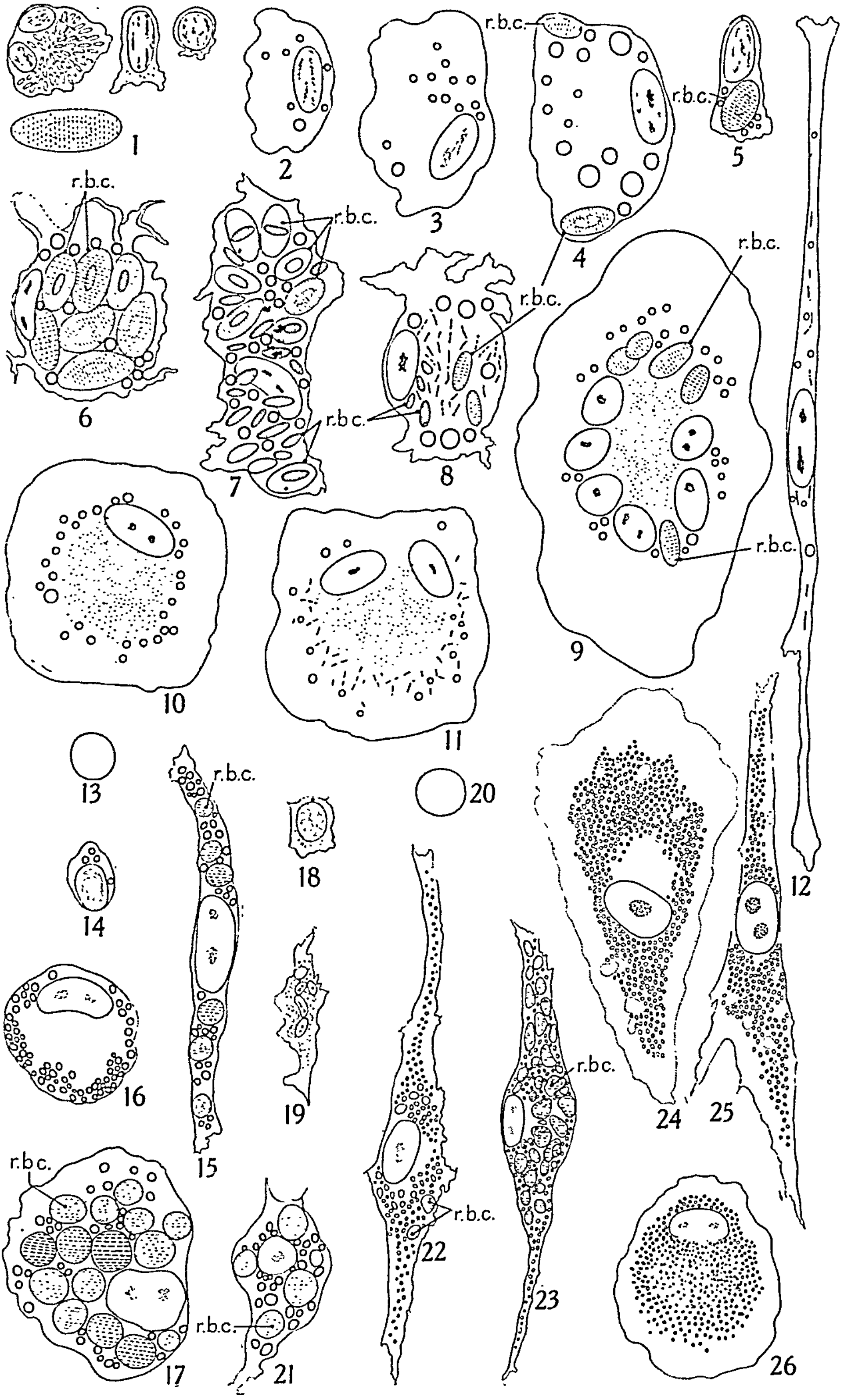
Fig. 22. A spindle-shaped cell from the human blood after 8 days' incubation.

Fig. 23. A cell of the fibroblast type, which, after it had grown eight days on celloidin, was again exposed to red blood-cells. It ingested many red blood-cells. These were partly laked and partly digested at the end of 10 days when the preparation was drawn.

Fig. 24. Epithelioid type of cell with enlarged centrosphere and large pale nucleus containing a nucleolus. Drawn after a drop of human blood had been incubated 13 days on celloidin.

Fig. 25. Spindle-shaped cell from human blood incubated 9 days.

Fig. 26. An epithelioid type of cell, showing the enlarged stained centrosphere; drawn from human blood, incubated 8 days on celloidin.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STUDIES ON BLOOD FIBRIN *

ITS QUANTITATIVE DETERMINATION; NORMAL FIBRIN VALUES, AND FACTORS WHICH INFLUENCE THE QUANTITY OF BLOOD FIBRIN

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Introduction. The origin of fibrinogen has been credited to various tissues of the body. Brown-Séquard,[1] Dastre,[2] Mathews [3] and others concluded that it was formed in the coats of the intestine. On the other hand, Doyon and Gautier [4] came to the conclusion that the intestine played no part whatever in the formation of fibrinogen. Goodpasture [5] decided that while the intestines were not essential to its production, they contributed to its formation. The lungs and the skin have also been considered seats of origin of fibrinogen.[2] Müller,[6] Morawitz and Rehn,[7] and others decided that it was formed in the bone marrow or by the leucocytes. This was supported by the high fibrinogen content of the blood in leucocytosis and in septic conditions, and by myeloid changes observed in the bone marrow, spleen, and liver following defibrination. So much for the extra-hepatic origin of fibrinogen.

Of particular interest is the evidence which has been accumulated to show that the primary seat of fibrinogen production is the liver. In 1894 Corin and Ansiaux,[8] and a little later Jacoby,[9] showed that the incoagulability of the blood in phosphorus poisoning is due to a disappearance of the fibrinogen. These observations were later definitely associated by Doyon, Morel and Kareff [10] with retrograde changes in the liver. At the same time Doyon [11] showed that large doses of chloroform by mouth caused a marked drop in the fibrinogen. This was also associated with pronounced changes in the liver. A drop in the fibrinogen was also observed by Whipple and Hurwitz[12] following prolonged chloroform anesthesia which produces a fatty degeneration and central necrosis of the liver. They observed not only a drop in the fibrinogen following the anesthesia, but a restoration to the normal level after the liver had regenerated some of its lost tissue. Meek[13] found that there was no regeneration of fibrino-

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gen after defibrination in animals in which an Eck fistula had been produced and both portal vein and hepatic artery had been ligated. On the contrary, the fibrinogen which remained in the blood rapidly disappeared. Recent clinical observations also point to the hepatic origin of fibrinogen. Isaac-Krieger and Hiege [14] and McLester and Davidson [15] observed a decrease in the fibrinogen in diseases of the liver, which involve destruction of the parenchyma, including acute yellow atrophy, carcinoma and tuberculosis of the liver.

Observations indicating the rôle of the liver were also made recently by Schultz, Hall and Baker [16] in connection with experimental necrosis and repair of the liver. They produced extensive infarct-like lesions in the liver of dogs by injecting small doses of chloroform into the portal system and followed the repair of these lesions. A marked prolongation in the clotting time of the blood was noted in many of the animals a day or two after the chloroform was administered. Indeed, some of the animals died of hemorrhage despite the precautions which were taken during the operations to ligate all bleeders. The peritoneal cavity usually contained large quantities of unclotted blood. There were also signs of considerable external hemorrhage. In the later stages of repair, three or four weeks after the lesions were induced, and when hyperplasia and hypertrophy of portions of the liver were usually marked the opposite was true. The blood now clotted with extraordinary rapidity, and clung to the autopsy surgeon's hands in long shreds. The studies reported in this paper are the outgrowth of the interest which these observations stimulated.

I. *Method of Determining the Fibrin.* Quantitative methods for the determination of blood fibrin have been reviewed by Robertson,[17] Gram,[18] Foster and Whipple,[19] Howe [20] and especially by Starlinger.[21] It is agreed that the older methods are unsatisfactory. Recent methods, which compare favorably with each other in accuracy, are those of Cullen and Van Slyke,[22] Gram,[18] Foster and Whipple [19] and Wu.[23] They are all alike in that the clot is obtained from the citrated or oxalated plasma by the addition of calcium chloride, but differ in the final steps. Wu determined the fibrin by colorimetric procedure. Cullen and Van Slyke determined it indirectly by the Kjeldahl method, calculating the amount of fibrin from the amount of N recovered. Gram washed the precipitated fibrin with distilled

water, alcohol and ether, dried and weighed it directly. Foster and Whipple dried the fibrin to a constant weight in an oven at 110° C., burned it and weighed the ash, and from the difference calculated the quantity of fibrin. The method of Foster and Whipple is probably on the whole the most satisfactory method, and more fully stated is as follows:

Approximately 9 c.c. of blood are delivered into a graduated centrifuge tube containing 1 c.c. of a 1 per cent sodium oxalate solution. The samples are then centrifuged for 30 minutes to bring down the cells. The cells and plasma are read to tenths of a cubic centimeter. Exactly 2 c.c. of the oxalated plasma are transferred to a "tumbler" containing 40 c.c. of 0.8 per cent NaCl and 2 c.c. of 2.5 per cent $CaCl_2$. This is allowed to stand at room temperature for 2 hours to flocculate the fibrin. The fibrin is freed from its saline bath by gentle manipulation and pressure with a glass rod and is then transferred to a crucible and dried at 110° C. for 3 to 10 hours, or until a constant weight is obtained. The protein is burned, the ash weighed, and from the difference in weight the amount of fibrin is calculated in terms of milligrams per 100 c.c. of plasma and of blood.

This method with two modifications was employed in all of the studies reported below. The first modification is in the amount of sodium oxalate used as an anticoagulant and the second is in the manipulation of the coagulum after recalcification.

In commenting on the amount of sodium oxalate which they employed, Foster and Whipple make the following statements: "The use of 1 c.c. of a 1.0 per cent solution with 9 c.c. of blood is close to the limit of safety. The addition of a little more blood to this mixture will often start blood coagulation." Preliminary studies soon satisfied us that this amount of sodium oxalate is altogether too close to the limit of safety for uniformly accurate results. We finally decided to use 1 c.c. of a 2 per cent solution to 9 c.c. of blood, and all of the figures given below are based upon this amount. By computation a 1.5 per cent solution of sodium oxalate is approximately isotonic with blood. A 2 per cent solution is therefore slightly hypertonic. However, when added in amounts of 1 c.c. to 9 c.c. of blood this degree of hypertonicity is not sufficient to influence appreciably the cell hematocrit readings.

To recover the fibrin from the oxalated plasma we proceed as follows: The "clotting solution," containing 0.8 per cent sodium

chloride and 0.125 per cent calcium chloride, is made up in bulk. It is siphoned off in 40 c.c. quantities into 50 c.c. centrifuge tubes, to which 2 c.c. of the oxalated plasma are added. Flocculation of the fibrin is secured in 30 minutes at 37° C., or within 1 to 2 hours at room temperature. The coagulum is manipulated as suggested by Foster and Whipple, but instead of lifting the water logged mass out of the tube, and drying it for 3 to 10 hours, it has been found possible to bring the coagulum down to a relatively firm, button-like mass by 5 minutes' centrifugation at high speed. This contains much less water and dries to constant weight in 1 hour at 110° C.

All determinations were made on duplicate samples of blood; that is, instead of making duplicate determinations on one sample, as was practised by Foster and Whipple, determinations were made on two samples of blood. This, we believe, is more in accord with acceptable analytical methods, though more blood is required.

The fibrin values in this paper are expressed in mgm. per 100 c.c. of blood, and were calculated by means of Gram's formula (24), which is given below (letters are ours):

$$\frac{(B - C) \times F \times 100}{(B - O) \times 2} = \text{mgm. fibrin in 100 c.c. of blood.}$$

In which,

B = total oxalated blood
C = total volume of cells
$(B - C)$ = total oxalated plasma
F = fibrin in 2 c.c. oxalated plasma
O = volume of oxalate solution

Using 1 c.c. of sodium oxalate solution this may be reduced to

$$\frac{(B - C) \times F \times 50}{B - 1} = \text{mgm. fibrin in 100 c.c. of blood.}$$

With few exceptions the blood samples were all obtained by heart puncture according to the method described by Schultz.[24] Samples of venous blood, the fibrin content of which was compared with that in arterial blood, were obtained from the jugular vein. Most of the animals were bled while under ether anesthesia. A careful study on several animals showed that ether anesthesia does not appreciably influence the amount of fibrin. Essentially the same fibrin values were obtained whether the animals were bled under anesthesia or

without anesthesia. Neither did prolonged ether anesthesia seem to exert any influence.

Dogs were used in all of the experiments. They were carefully selected as to health and vigor, and separated from the stock animals. They were kept in a large room which was supplied with additional heat during the cold nights. They were kept well bedded with shavings, and were carefully watched for signs of distemper. Small box kennels were provided for the animals following operations, where they were kept until they were able to hold their own among the other dogs.

The first group of animals on which experiments were conducted were kept on a diet of table scraps, but the majority of the animals were fed on Spratt's dog biscuits. Dog biscuits were decided on chiefly because we wanted to keep the animals on food of uniform composition. By analysis [25] these biscuits contain 18.9 per cent protein; 3.7 per cent fat and 56 per cent carbohydrates. The dogs were fed immediately after the samples were taken in the morning, and again about 4 P.M. Determinations were made daily on most of the animals.

II. *Normal Fibrin and Hematocrit Values.* Probably the most complete study of normal fibrin values in dogs has recently been reported by Foster and Whipple.[26] Animals fed on "liberal mixed diet" presented values ranging from 140 mgm. to 248 mgm. per 100 c.c. of blood. The average for the series (13 dogs) was *187 mgm.* per 100 c.c. of blood. Fasting animals gave values ranging from 121 mgm. to 219 mgm. per 100 c.c. of blood. The average for this series (10 dogs) was *167 mgm.* per 100 c.c. of blood. A series of determinations on one animal made at intervals of 3 to 15 days gave values ranging from 130 mgm. to 159 mgm. per 100 c.c. of blood. Diets rich in animal protein produced higher fibrin values, while fasting, fat or a carbohydrate diet tended to decrease the amount of fibrin.

Determinations carried out according to the method described above have given us somewhat higher fibrin values than those obtained by Foster and Whipple. Animals fed on a liberal diet of table scraps gave fibrin values ranging from 174 to 391 mgm. per 100 c.c. of blood (Table 1). The average of 29 determinations on 6 dogs was 255 mgm. per 100 c.c. of blood. The greatest individual variation was 36 per cent. This was in a dog on which fifteen daily

determinations had been made. The average individual variation was 15 per cent. This however includes animals on which only two to four determinations had been made. While the variation for the twenty-nine determinations was large (124 per cent), the mean fibrin for the individual dogs varied only 47 per cent. The average

TABLE I

Normal Fibrin and Hematocrit Values; Dogs on a Liberal Diet of Table Scraps

Dog No.	Number determinations	Fibrin Values				Hematocrit	
		Highest mgm.	Lowest mgm.	Mean mgm.	Per cent variation	Mean	Per cent variation
15	15	273	174	214	36.2	55.1	14.6
17	2	244	243	243	0.4	45.8	2.1
20	3	262	235	248	11.5	59.2	5.8
23	3	298	252	279	15.3	49.0	9.2
24	4	391	322	343	17.6	52.1	8.1
26	2	337	299	318	11.1	57.4	0.8

hematocrit reading was 54.2 per cent, and the average individual variation 6.7 per cent.

Still higher values were obtained on dogs fed on dog biscuits. The average of ninety-three determinations on 22 dogs was 308 mgm. per 100 c.c. of blood, or 21 per cent higher than the average for the dogs on a diet of table scraps. The fibrin values ranged from 200 to 441 mgm. per 100 c.c. of blood (Table 2). The highest average for individual animals was 385 mgm. while the lowest was 222 mgm. The former is 25 per cent above the average for the entire series, while the latter is 27 per cent below the general average. The averages of the remaining twenty animals varied less than 15 per cent from the general average. The variation in individual animals was considerably less than between different dogs. The average variation for the 22 dogs was 18.2 per cent. Five dogs gave individual variations of less than 10 per cent; twelve less than 20 per cent, and seventeen (or 77.5 per cent of the animals) less than 25 per cent. The two animals (Dogs Nos. 30 and 46) on which the largest number of determinations were made presented individual variations of 26 and 31 per cent respectively. The average cell hematocrit readings for the entire series was 53.9 per cent, and the average variation was 9.0 per cent. These figures are almost without exception based upon daily determinations.

In as much as these determinations were all made on arterial blood drawn from the left ventricle, and the determinations by previous workers were made on venous blood, a comparative study was made of the fibrin in arterial and venous blood. Duplicate samples of blood procured from the jugular vein were compared with duplicate samples obtained in the usual way from the left ventricle. Arterial blood throughout gave higher fibrin values. In the first animal (Table 3) the arterial blood ran from 5.4 to 22.9 per cent higher than venous blood; the average difference being 13.2 per cent. Attention is called to the rise in the fibrin which began on March 15th. This was due to an infection in the neck which followed the third bleeding. The fibrin thereupon rose 98 per cent in the arterial blood and 112 per cent in the venous blood. Attention should also be called to the drop in the cells which resulted from the daily re-

TABLE 2

Normal Fibrin and Hematocrit Values; Dogs on a Liberal Diet of Dog Biscuits

Dog No.	Number determinations	Fibrin per 100 c.c. blood				Hematocrit	
		Highest mgm.	Lowest mgm.	Mean mgm.	Per cent variation	Mean	Per cent variation
30..........	12	438	338	385	26.1	55.7	17.6
34..........	5	441	323	373	26.7	59.6	11.3
37..........	4	272	228	260	16.3	57.5	12.0
38..........	2	278	277	278	0.0	67.0	2.0
44..........	6	330	252	290	23.6	58.4	12.3
46..........	11	374	258	315	31.0	55.7	21.2
47..........	3	385	293	329	23.8	40.0	4.5
48..........	3	292	239	263	18.1	51.1	0.8
49..........	3	278	208	234	25.2	40.2	12.3
50..........	4	328	200	265	35.9	39.9	17.9
51..........	4	318	216	260	32.1	41.8	24.6
57..........	8	358	294	319	17.8	53.0	8.9
60..........	3	293	269	277	8.2	55.3	2.2
63..........	4	269	222	247	17.4	65.2	10.2
66..........	2	288	266	277	7.6	68.3	1.0
67..........	2	293	280	287	4.4	53.1	8.0
68..........	4	292	273	284	6.5	56.8	12.7
71..........	3	336	261	290	22.3	48.1	4.9
72..........	3	385	373	380	3.1	49.6	2.0
73..........	3	394	332	357	15.7	54.6	8.7
74..........	2	241	203	222	15.7	56.5	1.8
75..........	2	371	363	367	2.2	47.2	3.6

moval of 40 c.c. of blood. Determinations made on two other dogs likewise gave higher fibrin values in arterial blood. In one of the dogs the average difference was 7.3 per cent, and in the other 15.2 per cent. These results are in harmony with the observations which were made by Dastre [27] in 1893, but which seem not to have been confirmed. Dastre claimed that blood taken from the inferior vena cava furnished a noticeably smaller quantity of fibrin than did

TABLE 3

Showing the Difference in the Fibrin Content of Arterial and Venous Blood; Dog No. 60

Date	Weight kgm.	Arterial blood		Venous blood		Difference in favor of arterial blood	
		Fibrin	*Cells*	*Fibrin*	*Cells*	*Fibrin*	*Cells*
March		mgm.	%	mgm.	%	mgm.	%
12	19.5	269	56.3	255	55.5	14	5.4
13	19.3	293	55.6	262	53.0	31	11.8
14	19.5	269	53.9	230	54.3	39	16.9
15	18.6	354	52.8	288	54.9	66	22.9
17	18.3	535	51.3	487	53.3	48	9.8
18	17.7	537	48.5	477	49.2	60	12.5

arterial blood. It therefore appears that fibrinogen is rapidly consumed by the tissues and that it plays a manifold rôle in the economy of the body.

III. *Factors Influencing the Quantity of Blood Fibrin.* (A) Influence of liver necrosis. Reference has already been made to the alteration in the coagulability of the blood observed by Schultz, Hall and Baker after the injection of chloroform into the portal system. The studies reported in this paper were initiated primarily by these observations. While the influence of prolonged chloroform anesthesia and of phosphorus poisoning, in which generalized effects cannot be ruled out, had already been determined, there had been no studies made on the blood fibrin following the production of hepatic lesions of this type, in which little of the toxic agent reaches beyond the confines of the liver. If administered slowly one is rarely able to detect chloroform in the animal's breath. The effects on the liver, however, become manifest immediately. It becomes strikingly mottled, due undoubtedly to localized circulatory disturbances, though the action on the liver is toxic as well as me-

chanical. The lesions which appear are firm, raised and infarct-like, and range from about one millimeter to several centimeters in diameter. The amount of liver destruction is in a measure proportional to the amount of chloroform injected. One tenth of a cubic centimeter per kilogram of animal weight will, if injected slowly, destroy about a third of the parenchyma. If this dose is doubled, almost complete necrosis of the liver may result. Animals usually do not survive the larger doses.

In general, the decline in the fibrinogen was not so marked as we anticipated from the observations referred to above. The injection of 0.1 c.c. of chloroform per kilo of animal weight caused a drop of

TABLE 4

Showing the effect of 0.2 c.c. of chloroform per kilo injected into the portal system; dog No. 50

Date	Weight	Fibrin	Hematocrit	Remarks
Feb. 11	6.1	200	44.1	Condition normal
" 12	5.7	220	40.8	" "
" 14	6.1	323	38.4	" "
" 15	6.3	328	36.2	" "
" 15 (10.45 A.M.)	....	....	....	Injected 1.2 c.c. $CHCl_3$
" 15 (3.45 P.M.)	....	362	31.8	Very sick
" 16 (8.00 A.M.)	....	22	24.8	" " Died soon after Almost complete necrosis of liver

from 30 to 50 per cent in 6 to 24 hours. By the 48th hour the fibrin generally returned to its previous level, where it sometimes remained for several days, when it rose distinctly above normal. This we attribute to the laparotomy wound, which we found difficult to keep free of infection. Eventually the fibrin returned to its normal level. Small doses of chloroform caused an immediate rise in the fibrinogen. Slight damage to the liver therefore stimulates fibrin production, as does tissue damage in other regions. Considerable damage to the liver seems to be necessary to bring the fibrin to a low level. Table 4 shows the results obtained on an animal injected with 0.2 c.c. of chloroform per kilo. The fibrin dropped from 328 mgm. to 22 mgm. in twenty-two hours. The liver showed almost complete necrosis. It is interesting that in this animal there was an initial rise in the fibrin at the end of six hours, in which time there is usually a well defined drop. We cannot account for this.

Three animals given 0.3, 0.4, and 0.5 c.c. of chloroform per kilo showed a decline in the fibrin within two hours. One showed a drop of 9.6 per cent at the end of the first hour; the second, a drop of 31 per cent at the end of one and a half hours; and the third, to our surprise, showed a rise of 38 per cent at the end of twenty minutes, which was then followed by a rapid decline. All of the animals died within three hours. In all of the animals the fibrin immediately after death was higher than in the previous determination, while the hematocrit reading was a little lower. We have made similar observations on other animals immediately after death.

It should be borne in mind that all of the results in this series of experiments were influenced to some extent by the laparotomy wound, which tends to stimulate fibrinogen production. This we demonstrated on three healthy dogs by subjecting them to the usual operation. The fibrin rose immediately in all of the animals. One of the animals showed a rise of 22.5 per cent in 24 hours; another a rise of 24.6 per cent, and the third, 48.9 per cent. The wound in each case appeared absolutely clean, having been covered with iodinized collodion at the time of the operation. The fibrin continued high for about four days, and then returned to normal. Wound infection always produces a marked increase in the blood fibrin.

The influence of prolonged chloroform anesthesia on the quantity of blood fibrin has been worked out by Whipple and Hurwitz [12] and by Foster and Whipple.[28] They observed a rapid decline in the fibrinogen following a two to three hour period of anesthesia, a gradual restoration of the fibrinogen to its normal level and finally a distinct rise above the normal as the parenchyma was restored. Experiments on three animals have yielded similar results in our hands. The animals were kept under chloroform continuously for three hours. Two of the animals died within 24 hours. One of these presented a drop in fibrin of 38.3 per cent in 15 hours, and the other a drop of 69.0 per cent in 21 hours. Both showed marked central necrosis of the liver. The third animal (Dog No. 57) survived and yielded some interesting figures (Table 5). The fibrin of this animal dropped from an average level of 318 mgm. per 100 c.c. of blood to 28 mgm. in 45 hours, a drop of over 90 per cent. It is interesting that only two days were required for the fibrin to return to its normal level. Regeneration of the parenchyma is far from complete in

this time, so that we must attribute the rapid restoration of the fibrinogen to greater activity of the remaining liver cells. This also explains the rise above normal which appears soon after.

Interesting results were also obtained when large doses of carbon tetrachloride were given by mouth. Schultz and Marx [29] have shown conclusively that carbon tetrachloride is decidedly toxic for the

TABLE 5

Showing the effect of prolonged chloroform anesthesia; dog No. 57

Date	Weight	Fibrin	Hemato-crit	Remarks
	kgm.	mgm.	%	
March 8	20.4	335	51.4	Bled without anesthesia
10	20.2	312	53.6	" " "
11	20.2	304	55.8	" " "
12	20.2	300	56.2	Bled under ether anesthesia
13	19.7	335	51.8	" " " "
14	19.5	358	51.2	" " " "
15	19.5	294	51.3	" " " "
17	19.5	312	52.2	" " " "
18	19.9	....	....	3 hrs. anesthesia (8.45 to 11.45 A.M.)
18	19.9	323	55.3	4 hrs. after anesthesia
19	18.8	260	63.8	20 " " " Sick
20	18.3	28	56.0	45 " " " Very sick
21	18.3	197	51.3	74 " " " Sick
22	18.1	291	48.9	94 " " " Better
24	18.7	357	46.3	Behavior quite normal
27	18.2	382	47.1	" " "
31	18.6	469	47.3	Active
April 3	19.4	291	45.1	"
5	19.7	266	49.1	"
7	19.3	272	45.7	"

liver, having an action comparable to that of chloroform. Doses as small as 0.05 c.c. per kilo of animal weight when injected into the duodenum produced in some animals outspoken fatty degeneration. Larger doses produced a well marked central necrosis. Similar doses given by mouth produced inconstant results.

Five normal animals were given 4 c.c. of carbon tetrachloride per kilo of body weight by means of a stomach tube, after a series of determinations had been made to determine their normal fibrin level, and the following results were obtained. Two of the dogs showed no drop whatever; two showed a drop of 30 per cent and 48

per cent respectively in 44 hours, at which time they were killed for histological study. The most striking results were obtained on the fifth animal (Dog No. 74). The blood fibrin in this animal dropped in twenty-four hours from its normal level of about 203 mgm. per 100 c.c. of blood to 88 mgm. In fifty-two hours, shortly before the animal was killed, the fibrin had dropped to practically nothing. The trace which presented itself when the plasma was recalcified could not be recovered for gravimetric determination.

The importance of these observations becomes manifest when the histological findings are described. The two animals which showed

TABLE 6

Showing fibrin values during infection

Dog No.	Number determinations	Fibrin per 100 c.c. blood			Condition
		Highest mgm.	Lowest mgm.	Mean mgm.	
16	19	709	357	470	Distemper and wound infection
17	4	512	402	455	Wound infection
19	7	659	324	444	Distemper
22	10	474	343	411	Granuloma on nose
23	7	750	416	507	Distemper and wound infection
27	12	538	411	459	" " " "
33	6	695	494	596	"
42	3	676	438	571	"
60	3	537	354	475	Cellulitis, neck
78	2	686	678	682	Distemper
80	5	480	396	437	"
81	2	820	806	813	"

no drop in fibrin showed no lesions whatever in the liver. The two animals which showed only a moderate drop in fibrin showed a fatty degeneration of the liver, with beginning central necrosis. The fifth animal, in which the fibrinogen had almost disappeared, showed almost complete necrosis of the liver. In some areas the lobules were completely destroyed, while in other areas only islets of liver cells were found surrounding the portal units.

(B) Influence of acute infections and suppuration. High fibrin values have been reported in acute infections and suppurative processes by Mathews,[3] Müller,[6] Morawitz and Rehn,[7] Foster and Whipple,[28] McLester [30] and others. McLester and Davidson [18] recently reported that they obtained low fibrin values in typhoid

fever, but high values in pneumonia and septic states. High fibrin values have been found not only in acute infections, or following the injection of bacterial products, but also following the production of sterile abscesses and other types of tissue injury. The termination of an acute infection, as the crisis in pneumonia, or the drainage of a sterile abscess, is followed by a rapid decline in the fibrinogen. Table 6 gives some values obtained on dogs with distemper, wound and other infections.

Summary

Normal fibrin values were obtained on 28 dogs, representing a total of 122 determinations. The average for animals on a diet of table scraps was 255 mgm. per 100 c.c. of blood, while the average for animals fed on dog biscuits was 308 mgm. per 100 c.c. of blood. Arterial blood yielded slightly higher fibrin values than venous blood.

Liver necrosis produced a decrease in the fibrinogen. Except when the damage to the liver was severe, the fibrinogen returned to its normal level within forty-eight hours. Chloroform injected into the portal system produced an immediate decline in the fibrinogen ranging from 30 to 90 per cent, depending on the amount injected and the length of time the animal lived. A similar decline followed prolonged chloroform anesthesia and carbon tetrachloride poisoning. One of the animals poisoned with carbon tetrachloride showed a complete disappearance of the fibrinogen. The liver of this animal had undergone almost complete necrosis.

Mild liver injury produced an immediate rise in the fibrinogen, as in the case of tissue injury elsewhere. The fibrinogen also rose immediately following a clean laparotomy. Acute infections produced the highest elevations. Ether anesthesia produced no noticeable effect.

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THE RELATION OF CHRONIC POISONING WITH COPPER TO HEMOCHROMATOSIS *

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Introduction. In a previous paper [1] two apparently unrelated subjects were presented, a histological study of the lesion of hemochromatosis in man and the results obtained from chronic poisoning with acetate of copper fed to rabbits. The present paper gives a summary of the former work, records certain aspects of the study of ten cases of hemochromatosis which came to autopsy at the Boston City Hospital within one year since then, and presents further experimental work with copper poisoning.

The main points of the first paper can be stated very briefly. In hemochromatosis a yellow pigment, hemofuscin, derived from hemoglobin, is deposited in the endothelium lining the sinusoids, and in the parenchymatous cells of the liver. In the course of time it is changed to hemosiderin. The transformation is very slow and requires at least months and probably years. When the parenchymatous cells are filled with pigment beyond a certain degree they undergo necrosis and the pigment is taken up by endothelial leukocytes which often collect in numbers, especially in the periportal connective tissue. Following necrosis, regeneration of liver cells occurs diffusely and in islands. The new cells in time become pigmented with hemofuscin which later changes to hemosiderin. Owing to the necrosis and disappearance of the liver cells, the stroma in places is relatively increased in amount by coalescence, resulting in sclerosis. In the foci where the liver cells regenerate, new stroma is formed as in a tumor. In this way the connective tissue in the liver is gradually increased in actual amount. It is possible to find all stages of the process terminating in pigment cirrhosis present in a section from a liver in which the changes are active.

Hemofuscin is also deposited later and more slowly in the fibroblasts of the stroma, especially around the larger blood vessels, in the smooth muscle cells of the arteries and veins and in the bile

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duct epithelium. In all these latter cells the hemofuscin is changed very slowly or not at all into hemosiderin.

Pigment appears early in the kidney, chiefly in the epithelium of some of the tubules, but never extensively. The cells killed by the deposit desquamate, are carried away by the secretion and replaced by regeneration. Neither accumulation of pigment nor sclerosis occurs.

After the liver cells have taken up about all the pigment they can hold it begins to be deposited in the other organs and tissues, especially in the pancreas, the cortex of the adrenal glands, the lymph nodes in the upper part of the abdomen, the heart, thyroid, skin of the extremities, mucosa of the stomach, etc. In the pancreas, necrosis, regeneration and sclerosis occur as in the liver; the physiological effect is to cause diabetes mellitus. Destruction of the cortex of the adrenal glands may lead to increase of the normal pigment (melanin) in the skin and other tissues, as in Addison's disease. Pigmentation of the heart may result in necrosis of muscle fibers and the formation of patches of sclerosis.

Chronic poisoning of rabbits with acetate of copper in small doses (100 mgm. or less daily on food) leads to the gradual deposit of a yellow pigment, hemofuscin, in the endothelium and parenchymatous cells of the liver. In three months' time necrosis and regeneration are active and many endothelial leukocytes are present filled with the pigment granules. In six to twelve months the animals die from sclerosis (cirrhosis) of the liver accompanied with jaundice. The hemofuscin shows slight or no change to hemosiderin.

The incidence of hemochromatosis. Hemochromatosis is usually considered a rare disease. During one year, March 1, 1922, to March 1, 1923, we had at the Boston City Hospital 288 postmortem examinations on adults. Ten of the bodies, 3.4 per cent, showed well-marked pigment cirrhosis with grossly evident deposit of hemosiderin in the upper abdominal lymph nodes and in the pancreas. Of the ten cases, four showed pigmentation of the skin, four had jaundice, two ascites, and one primary liver cell carcinoma. Only one, the last, was suspected of hemochromatosis during life and proved positive by excising a piece of skin and performing the iron reaction. All the other cases were accidental findings at the autopsy table. None had gone far enough for sugar to be demonstrated in

the urine. The patients did not die from hemochromatosis but from intercurrent complications such as lobar pneumonia, meningitis, etc.

Besides these ten cases of well-marked hemochromatosis there were three with a slight to moderate degree of pigment cirrhosis and nine showing hematogenous pigments in the liver, pancreas, heart and kidneys. They represented earlier stages or milder degrees of the process. The diagnosis in each instance was based on the presence of the two pigments characteristic of the disease, namely, hemofuscin, which was found practically only in the stroma, especially around the larger blood vessels, and hemosiderin, which occurred chiefly in the parenchymatous cells. In pernicious anemia much hemosiderin is often present in the liver and other organs, but hemofuscin does not occur except in an occasional case complicated with a slight degree of hemochromatosis.

In the year following this run of ten cases of well-developed hemochromatosis, under identical conditions and with interest unabated, no well-marked case came to autopsy.

Regeneration. One case, A23.48, in this series is of much interest because the process is active and in consequence the liver shows many small foci of liver cells, up to 2 mm. in diameter, at various time periods following regeneration. The cells in some foci contain no pigment, in others only hemofuscin, while in still others hemofuscin and hemosiderin occur in varying combination as the first pigment is gradually being transformed into the second.

In the pancreas a similar deposition of hemofuscin in regenerated acinar cells and its transformation into hemosiderin can be seen.

In two cases occurring in earlier years (A17.8 and A19.24), the liver showed similar islands of regeneration with various stages of the process of deposit of hemofuscin and transformation to hemosiderin.

In the liver of another (A22.164) of the series of ten cases the foci of regeneration are much larger, measuring many centimeters in extent, and resemble somewhat the picture presented by a liver of acute yellow atrophy during the stage of regeneration.. The large amount of hemosiderin present in the heart and kidneys in this instance suggests a much more active process than is usually found.

These four cases with foci of regeneration enable one to follow clearly the deposition of hemofuscin and its transformation into

hemosiderin but the time required for the change cannot be determined.

Chemical examination of organs for copper. The chief interest in this unusual series of cases lay in the opportunity it afforded of finding out, if possible, if chronic poisoning with copper could be connected with them in any way. Previous experimental work had failed to demonstrate copper in the hemofuscin even in the livers of rabbits poisoned with this metal.

Chemical examination of the liver and in some instances of certain other organs gave the following results. The first two examinations were made by a cruder method which would not show minute amounts.

	Tissue	Amount	Copper
A22.64	Liver (most of organ)	....	0. mgm.
A22.67	Liver (most of organ)	....	0. "
A22.164	Liver and other organs	1086 gm.	3.87 "
A22.286	Liver and kidney	1045 gm.	20. "
U22.11	Liver and other organs	952 gm.	3.35 "
A23.48	Liver	588 gm.	0.87 "
	Bone	160 gm.	0.50 "

Examination of the livers from several controls yielded somewhat similar figures.

	Tissue	Amount	Copper
A22.274	Liver	1095 gm.	15.5 mgm.
A22.275	Liver (Addison's disease)	980 gm.	4.5 "
A22.289	Liver	1930 gm.	1.5 "
M.L.C.	Liver (Alcoholic cirrhosis) . . .	2450 gm.	50. "

These results seemed to demonstrate quite conclusively that this line of investigation would not be a profitable one and it was therefore discontinued.

The cause of hemochromatosis. A careful study of the clinical histories [2] of the series of ten cases and also of the others occurring in previous years was made in order to find out what light they might throw on the cause of hemochromatosis. Unfortunately the records were found often inadequate or even wholly wanting owing in part at least to some of the patients having been brought in unconscious or in a dying condition without family or friends appearing.

Only two factors seemed to have any definite bearing on the pro-

duction of the disease, excessive indulgence in alcohol and contact with copper, chiefly through occupation. Some individuals had been exposed to both.

Two of the ten cases, A22.28 and U22.4, both females, 65 and 60 years of age, have entirely negative histories, except that one was stated to be "a good liver." With them belong two living cases, males who have cirrhosis of the liver, diabetes and pigmentation of the skin. Repeated questioning has failed to reveal any source of poisoning by copper.

Alcohol. Excessive indulgence in alcohol has frequently been noted as a possible etiological factor in the production of hemochromatosis. In this series of ten cases at least two, A22.164, male, 49 years, ship-builder and A22.315, male, 56 years, painter, had both used alcohol to excess for many years.

In the twenty-five years preceding the occurrence of these ten cases there had been seven cases of whom three, U02.39, female, 31 years, A19.24, male, 44 years, and A21.25, male, 55 years, are all stated to have drunk alcohol to excess. Two others, A15.117, male, 50 years, importer of Italian liquors, and A05.21, male, 84 years, longshoreman, are noted to have been alcoholic although one of them is said not to have been a drunkard. Still another, A17.8, male, 42 years, teamster, with typical and marked lesions of hemochromatosis complicated with slight alcoholic cirrhosis, had also drunk to excess.

One case of hemochromatosis, M1010, male, 51 years, occurring at the Peter Bent Brigham Hospital this past year is of much interest in this connection. The patient came in for treatment of hemorrhoids. He was found to have an enlarged liver, sugar in his urine and marked pigmentation of the face, neck and extremities. Examination of an excised piece of skin showed an abundance of yellow pigment in the corium, around the sweat glands and in the underlying fat tissue. The reactions for iron were strongly positive, indicating hemosiderin.

The man had been a bar-keeper for four years previous to National Prohibition and had consumed about one pint of whiskey and four to five glasses of beer daily. For the past six years, since the enforcement of prohibition, he had been a boot-legger and had also run a private copper still of his own, making about two gallons of corn

whiskey at a time in a tin-lined copper still, but with the coils in the condenser made of pure unlined copper.

Copper in alcoholic beverages. The occasional occurrence of lead in liquors, especially of the "moonshine" variety, in sufficient quantity to cause symptoms of acute lead poisoning suggested that copper also might be found. A limited number of chemical examinations gave the following results.

In six different wines the highest amount of copper to the liter was 1.68 mgm., in four fortified wines 0.89 mgm. In seven out of eight distilled liquors, chiefly of the variety known as "hooch," the amount in a liter varied from a trace to 10 mgm., but in the eighth it reached 185 mgm. A sample of "home-brew" made and drunk by a patient with cirrhosis of the liver and ascites gave 25.5 mgm. of copper to the liter. In the liquor removed from a still seized by the Boston police it amounted to 1250 mgm. to the liter (this would equal 4.95 grams of copper sulphate). This sample indicates the action of the acids in the ingredients to be distilled on a copper container. The copper salt would not, of course, distil over, but the volatile acids (chiefly acetic and citric) passing over with the alcohol during distillation would act in the same way on the copper coil or worm of the condenser and lead to the presence of more or less copper in the resultant liquor. This is evidently the way in which copper gains entrance to distilled liquors.

It was found that the ferrocyanide of potassium test for copper could be used perfectly readily on liquors. The only effect of the alcohol is to cause the precipitate of cupric ferrocyanide to occur in a flocculent form and to settle readily. Amounts as low as 10 mgm. to the liter were recognizable. By concentration through evaporation smaller amounts can be detected. The method furnishes a simple way of spotting liquors containing considerable copper and gives some idea of the amount. By the use of this method the Department of Public Health of Massachusetts was able to detect copper in nine out of eighty-four samples of "hooch" examined. The amounts present in five of the samples ran approximately 6, 10, 20, 25 and 26 mgm. of copper to the liter. The other four varied from a trace to 3 mgm. For exact determination quantitative methods must be used.

Two of the fortified wines contained lead to the amount of 53.0 and 74.1 mgm. to the liter.

The frequent association of alcoholic hyalin with pigment cirrhosis suggests that the cause of these two different types of lesions may often be contained in the same beverage.

Occupation. An attempt was made to find out if exposure to copper in consequence of occupation had any bearing on the production of hemochromatosis. The results obtained are interesting and suggestive but not definite and conclusive. Four of the ten cases had come in contact with copper for many years, A23.48, male, 63 years, an alcoholic, had worked at a lathe for two years milling and planing brass and then had continued for thirty-six years working on high grade steel in the same large dusty room. A22.67, male, 55 years, forged metals for twenty-five years in a dusty railroad shop where copper was handled. M941, male, 55 years, an alcoholic, polished pipe wrenches for thirteen years in the same place where brass pipes were milled and worked. A23.37, male, 46 years, fixed cables and telegraph wires as a lineman for twenty-three years.

One other case which came to postmortem examination at another hospital has a much clearer history of exposure to copper dust without alcohol playing any part in the etiology. U22.11, male, 41 years, had worked for fourteen years in a shop "milling and turning copper and brass" and then for two years had served as a plumber's clerk.

Three of our hospital cases dying of other diseases are of interest in this connection, although they had no pigment cirrhosis. A24.110, male, 49 years, worked in a brass foundry for eight years. A24.124, male, 60 years, was a copper worker since the age of fifteen. A23.19, male, 52 years, came to this country sixteen years ago. For six years he worked in a brass foundry, then, owing to brass (zinc) colic, he worked for the next ten years in an iron foundry. All three cases had the two characteristic pigments, hemofuscin and hemosiderin, in varying amounts in the liver, pancreas, heart and kidneys, chiefly in the fibroblasts of the stroma around the larger blood vessels. The first two had slight pigmentation of the extremities and skin excised during life gave a moderate but typical iron reaction.

In view of the apparent relation between exposure to copper dust and hemochromatosis suggested by this series of cases it was de-

termined to try to find out by animal experimentation what the pathological effect of swallowing or inhaling copper powder would be.

Copper dust. Copper dust inhaled may reach the lungs, or, in part at least, be swallowed with pharyngeal secretion or with sputum coughed up from the trachea. In the stomach the action of the hydrochloric acid theoretically at least should lead to the formation of a copper salt although text books on chemistry state that dilute hydrochloric acid has no effect on pure copper. Absorption of a copper salt should in time, if in sufficient quantity, result in pigment cirrhosis as in animals.

Fine copper powder affords a very simple and quick way of testing the effect of acids and alkalies on the metal, owing to the large amount of surface exposed. It was found that by shaking the powder vigorously in water or other fluids for ten to fifteen minutes a complete temporary suspension could be obtained with most of them. After standing a few minutes the copper settles to the bottom of the test tube and the supernatant fluid can be filtered. A series of experiments were tried.

The filtrate from a suspension of 200 mgm. of fine copper powder in 10 c.c. of distilled water gave no reaction with a two per cent solution of ferrocyanide of potassium. The filtrate from a similar suspension in a one per cent solution of nitric acid was pale greenish and gave an immediate heavy reddish brown precipitate with the same reagent. With hydrochloric acid the filtrate was colorless and gave a slowly forming whitish precipitate with the ferrocyanide. If, however, a minute amount of nitric acid or permanganate of potassium was added to the filtrate, to oxidize the copper salt present, or the filtrate was simply allowed to stand for twenty-four to forty-eight hours a strong characteristic precipitate occurred on adding the ferrocyanide. The filtrate from a suspension in blood serum gave a well marked reaction. These experiments demonstrate that copper powder dissolves to more or less extent in various acid and alkaline solutions.

Animal experiments. Five rabbits were given copper powder (100 to 200 mgm. to each animal) on their food daily. In two months' time two of the five died with pigmentation and beginning necrosis

of liver cells. The pigment occurred as yellow granules in liver cells and in endothelial leukocytes, most abundantly at the peripheries of the lobules, and in sections of fixed tissue stained slowly but deeply with basic aniline dyes (fuchsin and methylene blue), a characteristic of hemofuscin. Similar granules were found in the endothelium lining the sinusoids of the liver, the capillaries of the heart, in some of the renal cells and in fibroblasts around the larger blood vessels in the liver and kidneys. Examination of the feces in the large intestine showed only the coarser granules of copper to be present.

To test the action of the alkaline fluids of the body on copper, so as to be able to form an idea of what would happen to copper dust carried into the lungs, 100 mgm. of copper powder suspended in a two per cent solution of gelatine were injected subcutaneously in a rabbit, which was killed at the end of six days. There was extensive necrosis and inflammatory reaction at the site of injection. The adjoining skeletal muscle fibers were necrotic, often calcified and were surrounded by foreign body giant cells. The copper particles had all been dissolved except the coarser ones and they were surrounded by yellowish crystalline material. Hemofuscin granules in large numbers were present in the liver cells and endothelium, in many of the renal cells and abundantly in the capillary endothelium of the heart. They occurred in smaller numbers in fibroblasts around the larger blood vessels of the liver and kidney while the fibroblasts and endothelium of the bone marrow were filled with them.

A rabbit injected intravenously with 50 mgm. of copper powder in a gelatine suspension and with 100 mgm. on the next day, died in thirty-six hours. Delicate hemofuscin granules were already present in the liver cells, especially at the peripheries of the lobules, and to a slight extent in fibroblasts around the larger blood vessels.

Twelve milligrams injected intratracheally into a rabbit, which was killed at the end of ten days, caused necrosis of lung tissue with acute inflammatory reaction. The liver contained considerable hemofuscin in the parenchymatous cells, in the endothelium of the sinusoids and to a slight extent in the fibroblasts.

These experiments demonstrate clearly that copper powder obtaining entrance to the body through the gastrointestinal or respiratory tract is readily dissolved and absorbed and causes the deposi-

tion of a yellow pigment, hemofuscin, in the liver, heart, kidneys, bone marrow and probably other organs. The same is true of copper powder injected intravenously and subcutaneously.

Because the solution of gelatine was found to be slightly acid in reaction some of the experiments were repeated with a suspension of the powder in distilled water; identical results were obtained.

Copper in foods. One of the ten cases, M932, male, 50, worked eighteen years in a cannery cooking fruit in copper kettles. The question naturally arose as to whether he could have acquired chronic poisoning with copper owing to the nature of his occupation.

The presence of copper in foods does not seem to have attracted much attention as yet beyond the recognition of its use to color pickles and canned peas and beans. It is a problem for the chemist to attack.

Tests with several organic acids in one per cent solutions showed that citric, acetic, lactic, tartaric and tannic acids readily attack powdered copper. The most powerful is citric which equals nitric, the recognized solvent of copper. Acetic comes next and the others range below it. Sweet cider (malic acid?) has a similar effect. Because apple butter is regularly made in copper kettles, sweet cider was allowed to stand in a clean, bright copper kettle for twenty-four hours. A well marked reaction was obtained with ferrocyanide of potassium while the control was negative. It seems probable that acid fruits cooked in copper kettles are to be regarded with suspicion, and careful chemical examination should be made of those which, like apple butter, grape jelly and tomatoes, are most likely to be contaminated by cooking in copper kettles.

Copper kettles, coffee pots, water pipes, frying pans, etc. Three of the ten cases studied had used copper kettles for boiling water for cooking purposes. One of them had also worked at a copper occupation and a second had imbibed alcohol to excess. The third, however, had led an outdoor life and the use of alcohol was denied.

The filtrate from tap water boiled with copper powder in it gives no precipitate with ferrocyanide of potassium but there is other evidence of the action of tap water.

The hot and also in some instances cold, water pipes put into many houses in Brookline and other towns and cities years ago

were of brass. In the course of fifteen to twenty years they became so eroded that a pin often could be thrust through them and they had to be replaced with iron pipes. Evidently substances in the water had gradually dissolved both the copper and the zinc.

Copper and brass teapots are sometimes used; copper coffee pots are usually lined with silver, nickel or tin, but the lining is often removed through industrious scouring by cooks; both utensils, owing to the action on them of the tannic acid contained in tea and coffee, represent a possible source of chronic poisoning with copper. The same is even more true of similarly lined cocktail shakers because of the citric and other acids often present in the ingredients; they act on tin as well as on the copper. Beer requires investigation because one step in the process of making it is to pass the wort to the "copper" (large copper kettle or boiler) where it is boiled with hops.

The popular gas hot water heaters of the present day also may be a source of danger if the water passing through them is used for cooking or drinking purposes.

No positive evidence against this class of copper utensils and pipes has as yet been obtained, but they should all be regarded with a certain amount of suspicion. While the danger of poisoning from these sources is probably very slight it must be borne in mind because the possibility exists.

The filtrate from melted lard shaken with copper powder for fifteen minutes is bright bluish green in color owing to the formation of oleate and possibly other fatty acid salts of copper. Melted palmitic and stearic acids are colored blue in the same way. These experiments illustrate the danger of frying and cooking fatty substances in copper pans and kettles. This source of poisoning must be considered very real.

Incidence of hemofuscin in liver and other organs. The liver, pancreas, heart and kidneys were examined from all autopsies over a period of two and a half years in order to determine the frequency of occurrence of hemofuscin in them. It was found only in adults, of whom the youngest was forty-five and the oldest eighty-three. In 463 adults it occurred 54 times or in 11.6 per cent. It was always associated with hemosiderin although the latter was sometimes present only in a small amount. The hemofuscin was found chiefly

in fibroblasts where the connective tissue was most abundant, especially around the larger blood vessels. It is in this location that hemofuscin is changed most slowly or not at all to hemosiderin and therefore persists more or less indefinitely because of its insolubility. In parenchymatous cells it is gradually changed to hemosiderin, which is slowly dissolved and finally eliminated so that these cells may eventually become free of pigment if no fresh deposit occurs.

Four recent cases of atrophic cirrhosis (A23.109, male, 78; A23.144, male, 54; A24.3, male, 65; and A24.25, male, 74) are of interest in this connection. They are all evidently old cases of hemochromatosis in which pigment deposit has long since ceased so that reparative changes have been going on for years. Most of the hemosiderin has disappeared, but a certain amount along with hemofuscin is still present in the liver, pancreas, heart, kidneys and adrenals.

The significance of these findings is that ingestion of copper (if it is the cause of hemochromatosis) must be fairly common, that the body can handle and eliminate a certain small amount without injury to the organs, but that if the amount exceeds a certain minimum and is taken in over a long period of time, lesions are produced which may eventually cause the signs and symptoms of the disease known as hemochromatosis.

Hemofuscin. The nature of hemofuscin is not easy to ascertain, but it is probably only an intermediate product between hemoglobin and hemosiderin with properties different from either. It is preserved by all fixatives, is insoluble in water and dilute acids and stains deeply with basic aniline dyes while hemoglobin stains with acid dyes and hemosiderin with neither. The transformation of hemofuscin into hemosiderin can be followed in the regenerated foci in the liver of man and in the livers of experimental animals fed or injected with copper powder either by killing a series of them or by removing pieces of the organ surgically at various time intervals.

In some of the lower sea animals copper takes the place of iron in hemoglobin. It also forms a compound, cuprohemol, with hemol, a reduction-product of hemoglobin. In view of these facts and because poisoning with copper brings about the deposition of hemofuscin in the liver it would seem as though this pigment must contain copper. All the evidence obtainable thus far, however, is against

this view. It has not been possible to demonstrate copper in hemofuscin, even in that freshly formed in rabbits' livers, by either the hematoxylin or the triple nitrite (K, Pb, Cu) test.

Chronic poisoning with zinc salts causes a deposit of hemofuscin in the liver just as copper does and the same type of lesion: therefore, copper evidently is not necessary in order to form hemofuscin.

A rabbit injected subcutaneously with 0.75 gm. of copper powder suspended in distilled water died at the end of three days. Besides early but well-marked pigmentation in the liver there was also central necrosis of this organ, indicating the toxicity of the soluble copper salt formed by the dissolving action of the lymph. In addition numerous hemoglobin casts were present in the tubules of the kidneys.

Sheep are extremely sensitive to poisoning with copper and are killed by doses no larger than those effective with rabbits. They die not from the pigmentation of the liver, which is well-marked, but from occlusion of the renal tubules by hemoglobin casts.

This occurrence of hemoglobin casts in the kidneys in these experiments demonstrates destruction of red blood corpuscles and the setting free of a large amount of hemoglobin which evidently is not bound to copper. A slight degree of anemia has often been noted in patients afflicted with hemochromatosis.

In old hemorrhages, such as occur as the result of menstruation in chocolate cysts of the ovary due to uterine implants, where the hemoglobin is slowly transformed into hemosiderin, an intermediate yellow compound is formed which occurs often abundantly in small and large granules in fibroblasts and endothelial leukocytes. These granules stain deeply with basic aniline dyes and in other ways react like hemofuscin. This occurrence would seem to demonstrate beyond question that hemofuscin is only an intermediate product between hemoglobin and hemosiderin. Even better evidence on this point was afforded by injecting the hemoglobin obtained by the ether method from 25 c.c. of rabbit's blood into a medium sized rabbit. In twenty-four hours the liver cells contained numerous coarse granules of hemofuscin and the renal cells many fine ones.

Perhaps all that chronic poisoning with copper does is to cause a slight but persistent destruction of red blood corpuscles with setting free of hemoglobin.

The pigment deposited in the liver and other organs as the result of the action of copper does not at first stain readily with basic fuchsin. Hours instead of minutes are required. The granules in the endothelium lining the sinusoids are the first to stain deeply. Those in the liver cells follow much later. This difference in staining reaction would seem to indicate that the pigment which is first deposited slowly undergoes some transformation before it acquires the properties of hemofuscin.

Time necessary to produce hemochromatosis. Two questions are of interest under this heading. How long a time is required to change hemofuscin to hemosiderin and how many years does it take to produce the disease known as hemochromatosis?

Hemofuscin changes to hemosiderin very slowly in the rabbit: two to three years are required before granules begin to give a good reaction. In the sheep the process is more rapid so that hemosiderin can be demonstrated in less than a year. In a small South American monkey, as already reported,[1] the change was easily evident at the end of five months. The change in man is probably not any more rapid.

To produce a well-marked example of the disease hemochromatosis in all probability requires at least ten years and more likely fifteen to twenty or more. The great majority of cases are from forty to sixty years of age. The youngest listed here was thirty-one, and none younger has been found in the literature. The most definite history in regard to time is that of the man who "worked in a shop milling and turning copper and brass" for fourteen years and then lived two years longer.

Summary

Ten cases of hemochromatosis came to postmortem examination in one year at the Boston City Hospital; none was seen in the following year under identical conditions, hence the occurrence must be regarded as due to coincidence only.

Nine other cases occurring before or since this group of ten were also available for study, making a total of nineteen. Nine had used alcohol steadily for many years, usually to excess. Six were exposed to chronic poisoning with copper owing to their occupation (four in shops for thirteen to thirty-eight years milling and planing brass and

copper or inhaling the dust from these metals, one as lineman for twenty-three years for a telephone company, one in a cannery for eighteen years cooking fruits in copper kettles). One had regularly used a copper kettle for boiling water, three had entirely negative histories.

Chemical examination of the liver and of some of the other organs from several of these cases showed a small amount of copper but no more than was obtained from controls.

The cause of the disease seems to be connected chiefly with two different subjects, with alcohol and with an occupation involving copper.

Analyses of a limited number of distilled liquors gave from a trace to 185 mgm. of copper to the liter, evidently derived from the solvent action of organic acids on the copper worm of the condenser.

The ferrocyanide of potassium test will give a visible reaction with distilled liquors containing from 10 mgm. of copper up. By concentration through evaporation smaller amounts can be recognized.

A man drinking daily a quart of whiskey containing 185 mgm. of copper to the liter will take into his system about a gram of copper a week, which is, proportionately, comparable to the amount required slowly to produce pigment cirrhosis in rabbits and monkeys.

Fine copper powder shaken up in one per cent aqueous solutions of nitric, hydrochloric, acetic, tartaric, lactic and citric acids for fifteen minutes was dissolved to a perceptible degree. Some of the filtrates were colored green and all gave strong characteristic reactions with ferrocyanide of potassium. Citric acid was apparently about as active as nitric; acetic and the other acids ranged below them. The action of "sweet cider" on copper was strong, while lard melted and shaken with copper dust for fifteen minutes was found to be bluish green when filtered, owing to the solvent action of oleic acid. Palmitic and stearic acids were colored blue in the same way.

Fine copper powder fed on food to rabbits was dissolved in the gastrointestinal tract and caused death in two months with marked pigmentation of the liver. Injected intravenously, intratracheally or subcutaneously it was quickly dissolved by the fluids of the body, causing marked local reaction and pigment deposit in the liver, kidneys, bone marrow and some of the other organs and in some instances death in thirty to seventy-two hours.

Chronic poisoning with copper in sheep and acute poisoning in rabbits caused, in addition to pigmentation, blocking of the tubules of the kidneys with hemoglobin casts, indicating destruction of red blood corpuscles.

Hemofuscin, one of the two pigments always present in hemochromatosis, is apparently nothing but an intermediate product between hemoglobin and hemosiderin. It is deposited in the liver and other organs of animals as the result of poisoning both with copper and with zinc, but it is also found in the liver and kidney following intravenous injection of hemoglobin and in the cells of the tissues surrounding old hemorrhages.

The change of hemofuscin to hemosiderin can be followed in the livers of animals poisoned with copper and also in the islands of regenerated cells in the liver of man.

Microscopic examination of the liver, pancreas, kidneys and heart in all adult human beings (463) coming to autopsy during two and one half years showed hemofuscin and hemosiderin present in fifty-four, or 11.6 per cent.

Conclusions

Evidence is steadily accumulating to prove that chronic poisoning with copper is the cause of hemochromatosis. While the fully developed disease is relatively rare, the early stages and the lighter forms are fairly common, but are necessarily unrecognized by the clinician and commonly overlooked by the pathologist.

The sources of the poisoning are (1) distilled liquors contaminated with copper dissolved from the copper worm of the condenser by the action of volatile organic acids (citric, acetic, etc.); (2) occupations involving exposure to copper dust (brass foundries, brass milling, planing and polishing, telephone line-repairing) as the metal is readily dissolved in the juices of the body however it may obtain entrance, and (3) probably also acid foods, jellies, candies, etc., contaminated by copper owing to having been cooked in copper vessels. Under this last heading must be included foods cooked and fried in copper kettles and pans owing to the dissolving action of oleic, palmitic and stearic acids in lard and other fats on the metal.

Experimental work with animals demonstrates that copper inhaled or ingested is dangerous to life although its action as a chronic

poison is exceedingly slow. This is the reason its deleterious effect has been so long overlooked.

Studies of clinical cases show that ordinarily it takes fifteen to twenty-five or more years to produce the symptom complex of the disease known as hemochromatosis.

Now that the danger of poisoning has been pointed out, steps should be taken to prevent copper getting into liquors and foods and to protect workers in occupations involving copper from inhaling or ingesting copper dust.

The reason that copper is so generally found in minute quantities in human organs is not due to its being a normal constituent of the tissues but because man is constantly exposed to taking the metal into his system through foods and drinks contaminated with it. While small amounts cause no harm, because slowly eliminated, larger amounts absorbed during many years are exceedingly dangerous. Susceptibility to poisoning by copper probably plays a part as with lead and arsenic.

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EVIDENCE OF REGENERATION OF PANCREAS IN AN INSULIN TREATED CASE OF DIABETES *

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The peculiar pathology of the pancreas renders a knowledge of its histology essential to the understanding of any disease process involving it. The absence of any gross changes in the pancreas in many cases of diabetes has led to much confusion respecting the pathology of this disease and to some doubt of its being of pancreatic origin. Current literature tends to the view that changes are present when a close study of the histopathology of the organ is made.

Early in embryonic life three buds from the primitive intestinal tract develop, two ventral (Santorini) and a dorsal one (Wirsung). By the end of the second month of fetal life these anlagen fuse and give rise to all the structures of the adult pancreas. Pearce [1] and Küster [2] note separation and vascularity of cell groups in the third month. Such groups consist of four more or less distinct types of tissue — the ducts, centro-acinar cells, islet cells, and acinar cells. These structures are arranged in lobes separated by small amounts of connective tissue and again subdivided into lobules by the ramifications of the ducts. A typical lobule consists of a semi-spherical mass of cells into the centre of which a duct enters. In close proximity to the duct and possibly arising from it are numbers of flattened clear cells, the centro-acinar cells. The remainder of the lobule consists of pyramid-shaped acinar cells whose apices are

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filled with zymogenous granules, and the so-called islands of Langerhans. These were first described by Langerhans[3] in 1869 as small groups of cells studding the pancreas and differentiated from the acinar cells by their clarity and lack of zymogen granules. As early as 1886, Lewaschew[4] observed the presence of two types of cells in these islets differentiated by their shape and mode of delimitation. Ten years later Laguesse[5] described the presence of safranophile granules resembling zymogen granules but finer and more resistant to acetic acid. From this period until 1907 when Lane's work appeared, little progress was made in the study of the histopathology of the pancreas, the literature during this period being chiefly concerned with the origin of islets and a discussion as to their being a histological entity at all. The work of Lane[6] and Bensley,[7] through elaboration of technique and stains, has made possible a much clearer understanding of the composite cells of the islets and has shown that the early workers, because of their inability to differentiate clearly the acinar and islet cells, had given a wrong interpretation of their nature. These workers showed the presence of two, and possibly three, types of cells in an islet, the so-called A and B cells, and a third less differentiated Gamma cell. The cells of the islet are typically arranged in cords, but in many instances solitary islet cells are noted among a group of acinar cells. Some of these it is claimed are the result of artifacts. Islets are frequently found in close association with ducts but never actually communicate with them. This fact and their great vascularity led to the belief that these cells produced an internal secretion.

The frequent lack of gross pathological change in the pancreas has been a stumbling block to the acceptance of the theory of the pancreatic origin of the disease. The so-called simple atrophy of the pancreas with diminution in the number of islets alone, and accompanied in older patients by interacinar fibrosis, has been reported often. Pleasants[8] in 1900 described six cases of this type in children, considering it an evidence of congenital lack of development of these structures. A little later Ssobolew[9] reported fifteen such cases, in four of which no islets at all were observed. Failure of these observers to make serial sections and the lack of suitable staining technique may explain many of these negative findings. Hydropic degeneration of the islet cells, now regarded as pathognomonic of diabetes, was first described by Weichselbaum and

Stangl[10] in 1911, and shortly after, Homans[11] showed that these changes were confined to the B cells. Allen[12] has recently made elaborate studies of this type of degeneration and the factors influencing its development. He found definite vacuolation of the cells within five days of partial pancreatectomy. The remaining islet cells rapidly degnerate after this operation and in six weeks to two months the B cells are completely destroyed and replaced by acinar tissue with small groups of A cells interspersed. While under these conditions, the majority of vacuolated cells die; this may be prevented by the avoidance of over-function by means of diet and may allow recovery to occur. Such islands always remain small and may readily be overlooked without the use of suitable stains, giving the appearance of simple atrophy with decrease in the number of islets. Allen has also shown that active diabetes is a prerequisite to the development of these changes.

The following case was found suitable for reporting because of the clinical response to insulin associated with anatomical changes strongly suggestive of regeneration in the pancreas.

Clinical History: B. N. white male — age 9.

Family History: Father and one maternal uncle both have diabetes.

Diabetes was diagnosed in this child when he was two years old. He was placed on a suitable Allen diet, which was strictly adhered to, and for a time did well except for recurrent attacks of dysentery, which lowered his tolerance. Failure to gain in stature or weight in any way commensurable with his age was noted and the general conditon became worse each year until he was more or less a chronic invalid with increasingly frequent attacks of acidosis during the last year before starting insulin.

He was admitted to the Hospital for Sick Children, Toronto, the end of December 1922. At this time he was an emaciated dwarf, more or less drowsy and unhappy. His weight was 30 pounds and his height 39 inches. His tolerance to carbohydrate had decreased until he was now unable to utilize 15 grams of such food. Insulin treatment was started at once and his diet increased to a diet suitable for a boy of his age, sufficient insulin being given to keep him sugar free and his blood sugar normal. He was discharged on an adequate diet plus insulin. Progress both in general condition and in improvement of pancreatic function was steady.

The accompanying chart shows his decreasing need of insulin with his gain in weight. His tolerance to carbohydrate trebled in the year as shown either by the fact that 30 units of insulin controlled the disease as adequately as 90 a year before, or, stated in another way, without insulin he could now handle 45 grams of carbohydrate in place of 15.

The photographs taken when insulin was started and six months later indicated in some measure the improvement in general condition. From a chronic invalid in 1922 he became the leader of the "gang" in 1923. He was killed by

fracturing his skull when sleigh-riding. He lived only about three hours after receipt of the injury and an immediate post mortem examination was made.

Autopsy Findings: The pancreas, which was removed within thirty minutes of death, was immediately cut into small blocks, labeled and portions from the head, body, and tail placed in a 10-per-cent solution of neutral formalin and also in Zenker's fluid with acetic acid. The blocks were embedded in paraffin and sections from 2–6 microns were cut.

The formalin-fixed sections were stained in Ehrlich's hematoxylin and eosin. Some formalin-fixed material was further fixed in Bensley's acetic-osmic-bichromate mixture as for fresh tissue and later stained with Altmann's anilin acid fuchsin and methyl green. Cresyl violet was also used with some of this latter material to bring out nuclear contents more clearly.

The Zenker-fixed preparations were stained with a variety of special stains to show up their specific granules. The stains used were Bensley's neutral gentian violet, Martin's azo-fuchsin and basic ethyl violet, Bensley's safranin-acid violet, Altmann's acid fuchsin and methyl green, Mallory's phosphotungstic acid hematoxylin, and finally a stain by Bowie, to whom we are indebted for the privilege of using the method before publication, namely Biebrich scarlet and basic ethyl violet. This latter stain we found very satisfactory as a differential stain showing up as it does, with the acid Zenker's fixative, the granules of the A cells blue and those of the B cells red.

The pancreas in the gross, as in so many cases of diabetes, presented nothing abnormal. It weighed twenty-nine grams, which, considering the age of the patient and his condition, was good weight. Its color, shape, lobulation, and consistency appeared normal. On gross section nothing indicating a fibrosis, adipose replacement, fat necrosis, or hemorrhagic necrosis could be detected.

On histological examination of sections from different areas we were unable to find any distinctive lesions such as hydropic degeneration of islet cells, fibrosis or atrophy of the islets as are commonly described associated with diabetes. Neither could we detect associated lesions such as inter- and intra-lobular fibrosis, chronic pancreatitis, simple atrophy, or adipose replacement. The number and size of the islets gave no indication of the existence of a possible diabetes. The only finding was the presence of occasional pyknotic

nuclei in some of the islet cells. The negative findings just mentioned, however, are not inconsistent with diabetes. This has been pointed out by many observers and serves to prove that functional capacity of the islet cells, as determined by clinical laboratory tests, is after all the final criterion for determining the ability of the individual to utilize carbohydrates. With our present facilities we are not able histologically to estimate function and we must therefore rely upon clinical tests for this determination. While clinically, in this case, there was a marked rise in sugar tolerance, it never returned to normal and the case therefore remained one of diabetes. The rise in sugar tolerance would indicate either that the islets were not irreparably injured, or if so, that regeneration had taken place in others to compensate for the loss.

While nothing in the nature of a retrograde process was found in this pancreas beyond the presence of a few pyknotic nuclei in some of the islet cells, one could recognize many evidences of regeneration of the acinar, centro-acinar, and islet cells. About the margin of the pancreas the lobular arrangement became quite apparent and, in fact, was exaggerated into papillomatous structures composed of a garland of acinar cells surrounding a group of centro-acinar cells and drained by one of the radicles of the pancreatic duct. In some of the larger lobules there were small groups or cords of islet cells associated with the duct or centro-acinar cells. Throughout, there appeared to be a definite increase in the number of centro-acinar cells. These were readily identified with Bowie's stain by their large, oval-shaped-pinkish staining nuclei, with single or double nucleoli, and very little if any chromatin material and clear cytoplasm. This hyperplasia of the centro-acinar cells was most apparent in the peripheral portions of the pancreas.

Our attention was then directed towards a possible anatomical explanation of the increased functional capacity of the islet system.

Hypertrophy of the islets has been observed under various conditions and has been credited with the prevention of glycosuria in such cases. Opie [13] questions the functional efficiency of these islets, as similar islets are frequently noted in association with diabetes, probably in response to functional overstrain. Such hypertrophy has been reported in diabetes by Cecil,[14] and unassociated with glycosuria in cirrhosis of the liver by Ohlmacher,[15] and about the advancing margin of malignant growths by Pearce.[16] McCallum [17]

reported two cases of diabetes in children in whom there were large islets not sharply outlined because of apparent continuation of the cell strands with the acini. The acini were also hypertrophied. Weichselbaum [18] frequently observed such changes and considered them as regeneration by the outgrowth of solid columns of cells from the ducts. It would be impossible to be certain of the nature of the cells in these large islets with the technique used by these authors.

By the use of special islet cell stains described above, the islets stood out clearly and gave one the impression of a distinct increase in number over those observed with the ordinary stains. This increase can be explained by the presence of a number of islets made up of cells with large nuclei and having a very intimate association with the acinar cells, which with the ordinary strains were very difficult to identify. With the special stains, however, more particularly Bowie's stain, the islet cells were easily identified and the characteristic staining of their granules noted.

The size, distribution, and number of islets varied considerably in different areas of the same section. Some were quite large, others small, often consisting merely of a cluster or cord of six or eight cells. As far as we could tell with the number of sections studied, the distribution of the islets throughout the whole pancreas was fairly uniform, with possibly a few more islets in the tail. What was more striking was the vast number of islets to be found in certain peripheral areas of the cross section of the pancreas as compared with the more central portions. In some peripheral areas one could identify as many as twelve to fourteen islets in a low-powered field of the microscope, whereas in the central portions we found only one islet in two and three low-powered fields. It is of course not unusual in a fibrosed pancreas, or in one showing adipose replacement, to find the islets closely packed together. In this case, however, both of these factors were absent.

We were also able to identify certain differences in the islets of the peripheral portions from those of the more central areas which lead us to believe that, broadly speaking, we were dealing with two types of islets. This distinction was not made upon characteristics of the individual cells, but rather upon their distribution, size, shape, structure, and percentage of A cells to B cells present. Neither is it suggested that they might be functionally different beyond the possible effect of a deficiency of A cells in the one group.

Throughout the central portions of the pancreas the islets as a rule were larger and contained more islet cells than one normally finds. Their cells were closely packed together and many of them showed pyknotic changes in their nuclei. They lacked the orderly structural arrangement in cords, and appeared jumbled. The outline of the islet was very irregular and islet cells could be seen extending from the main group into the surrounding acinar tissue for varying distances. No capsule, therefore, could be distinguished, although there appeared to be a normal amount of stroma supporting the individual cells. The ratio of A cells to B cells was approximately normal, the A cells representing from twenty to fifty per cent of the total number.

In the peripheral portions of the pancreas and in the centers of the finer pancreatic lobules we found islets having certain morphological changes which set them apart from those just described. As a rule they were not so large as those first described, often consisting of not more than six or eight cells. While they had no apparent capsule, they were well defined by the pronounced staining characteristics of their cells, which were also arranged in definite cords or well-defined round masses. Their cells appeared larger than the cells of the first type of islet. A small amount of connective tissue stroma supported these cells and in it were one or more blood capillaries. Their close association with the finer duct radicles and with groups of centro-acinar cells was quite obvious. In some cases they could be distinguished from centro-acinar and duct cells only by the specific staining of their granules. As an evidence of the more active growth of the islet cells we very often found them compressing the surrounding acinar cells into thin, irregular, deeply staining masses of cytoplasm. The most striking feature of these islets possibly was the almost entire absence of A cells. Occasionally we could find one or two A cells, but for the most part they were composed entirely of B cells, which were quite large with large, round or oval reticulated nuclei and which showed in their cytoplasm, with Bowie's stain, large numbers of reddish granules.

From these findings we were convinced that here we were dealing with islets which were possibly in age or time of origin different. The large central islets showing evidences of pyknotic degeneration and the lack of an orderly arrangement of their cells, and numerous A cells, we felt were older islets which existed prior to the insulin

treatment. Their increased cellularity and the spreading out of their cells into the surrounding acinar tissue suggested some attempt at regeneration.

The very numerous islets found in the peripheral areas we believe represent younger islets. This conclusion is based upon the larger size of their cells as compared with the former, their orderly arrangement in cords, absence of pyknosis, their very close association with groups of centro-acinar cells, and by the presence of practically the one type of cell, the B cell.

In view of the fact, as pointed out by Allen [12] and Homans [11] that the B cells are the antidiabetogenic cells and the ones first destroyed in severe diabetes, it is interesting to note in this case the almost complete dominance of B cells in so many of the peripheral islets as compared with those of a normal pancreas or of the central areas of this pancreas. These islets we believe are new islets which have probably developed since the commencement of insulin treatment and in functioning have been responsible in a measure for the rise in sugar tolerance. Their distribution and prominence in the peripheral portions of the pancreas suggest very strongly that they are new islets forming in the natural growth of the pancreas. The insulin treatment in relieving the strain placed upon the already functionally deficient islet system allowed the new islets to develop and assume their normal functional capacity.

Discussion by Prof. R. R. Bensley

I am very happy to be here today and to hear this case presented by Dr. Gladys Boyd. There is perhaps no problem so difficult for the pathologist to approach as that of explaining, on the basis of histological changes, why the patient improves. This case of pancreatic response to insulin treatment gives us new hopes, and increases the responsibility of the pathologist in the investigation of similar material. The interpretation of such changes as Dr. Boyd has pointed out to us is very difficult. In the absence of controls one can only imagine what the condition of the pancreas was prior to the insulin treatment, and attempt to read the process on the basis of this hypothetical condition. In Dr. Boyd's case I was more impressed with certain groups of small bulbous islands, clustered round a duct, which strongly resembled in morphology and arrangement the regenerative islands seen in experimental animals, than by

the other evidences of regeneration which she has enumerated. I have little doubt that these were actually new island units. In these cases the application of the vital-staining methods would undoubtedly render great service. I realize the difficulty of applying such methods to the study of the human pancreas, but it was accomplished successfully by Clark in several cases. The advantage of these methods is that they permit the intelligent inspection of the whole pancreas. It is the lack of such comprehensive study that is responsible for much of the confusion in interpreting the gland.

The literature of pancreatic regeneration is most unsatisfactory. A good deal is quite unreliable because of the difficulty of distinguishing between regressive and regenerative changes. The changes described by Kyrle are to my mind largely regressive. De la Roche, working under the direction of Laguesse, studied the events which followed the ligation of the pancreatic duct and found that there was considerable effort to reconstruct both acini and ducts. Some years ago I presented to the Harvey Society a description of some findings of Clark encountered in the study of similar cases of duct ligation. He found that, contrary to the usual opinion, acinous tissue did not disappear wholly until a long time after duct ligation, though the destruction of this tissue was extensive. It is fortunate that Banting supposed that the acinous tissue did disappear under these conditions, since we owe to this idea the discovery of insulin. What Banting had was probably a pancreas still containing abundant island tissue but reduced to its lowest ebb of zymogenic activity. After ligation of the duct the process was wholly destructive for a time, involving both islands and acinous tissue, but the latter more than the former. At the end of a month regenerative processes became dominant, again involving both tissues, and resulting in the formation of new acini and new islands. The newly formed acini, since they have no outlet by the duct, in turn succumb to the destructive process, but the new islands increase progressively until a maximum is reached.

In this series of experimental animals a few were encountered in which autopsy, several months after ligation of the duct, revealed a complete pancreas differing from the normal only in the fact that it was smaller in size than normal, and contained more fibrous interstitial tissue. Since this change was entirely confined to the pancreas, we interpreted these as cases in which a successful ligation

of the duct had been followed by the usual regressive changes, but in which the accidental reëstablishment of the communication of the duct with the bowel had permitted a complete regeneration of the pancreas. In one of these cases the duct had found a new opening at some distance from the stump of the old duct, which was identified at autopsy. This interpretation has been confirmed recently in my laboratory by my student Grauer, who undertook the task of reimplanting the duct into the bowel a month after a successful ligation of the duct. This reimplantation presents great difficulties and so far he had had only two successful cases out of about forty-five attempts. In every case controls of the pancreas are taken at the time of reimplantation. In one of these cases he found one month after reimplantation a complete pancreas similar in all respects to those observed by Clark. The control tissue from this animal taken at the time of reimplantation showed a typical one-month ligation picture. In the second case, examined two weeks after reimplantation of the duct, the acini were growing out into the fibrous envelope of the pancreas and covering the newly formed islets. On the basis of these experiments and observations it is my opinion that, given appropriate conditions, the capacity of the pancreas for regeneration is one hundred per cent. I believe that we shall ultimately learn how to control it. It is significant that in these observations the regenerative emphasis seems to be on the island tissue as long as the duct remains closed, but shifts to the acinous tissue as soon as communication with the bowel is reëstablished.

It is easy to see from these experiments that a pancreas obtained at autopsy in a case of diabetes may have passed through a series of kaleidoscopic changes which it would tax the imagination to figure out. For this reason great caution is necessary in the interpretation of such material.

The greatest dilemma of all in applying the insular hypothesis to the pathology of diabetes is presented by those cases in which no changes can be demonstrated in the pancreas. Various attempts have been made to explain these cases on a basis of numerical reduction of the islands, on reduction in size or in functional competency. I regret to say that I have little confidence in the results of enumeration of islands in sections. The amount of tissue which can be inspected by such methods is too infinitesimal to warrant

general conclusions. It must be remembered that we know very little about the mechanism of physiological control in the endocrine organs, and until we know more it is futile to speculate. It would be better to attempt no explanation at all of these difficult cases than to yield to the temptation to adapt the facts to the insular hypothesis. Meanwhile, such cases as the one presented by Dr. Boyd are of great importance, and will, no doubt, reward careful study by the pathologist.

Reply — Dr. Boyd

I would just like to read to you extracts from letters from Dr. F. M. Allen and Dr. Eugene Opie, giving their opinion on this case.

1. Dr. Allen: "I have the impression, first, that the islands are more numerous than should be anticipated in a child with the low tolerance which you mention before insulin treatment. Furthermore, some of the islands are large and typical, but small and irregular islands are more common than normal. They have the true capillary and trabecular framework, but the capsule is often not visible and they merge with the surrounding acinar tissue with appearances strongly suggesting 'transitions.' The special granule stain as usual excludes any true transitions between island and acinar tissue, and shows furthermore that the cytology of the islands is normal. It is altogether probable that some children at least should have the power of forming new island tissue. It has been rather surprising that this process, which must be a regular occurrence in the growing pancreas of the normal child, has been so deficient in diabetic children under diet treatment. From the combined clinical and microscopic evidence, I think you have grounds for the belief that new formation of island tissue has occurred in this case under insulin treatment. Presumably this growth has occurred by enlargement of existing islands or prolifition of ducts."

2. Dr. Opie: "I have received the second set of slides from your case of diabetic pancreas. I find in them very definite evidence of hypertrophy of the islands of Langerhans, with columns of cells extending into the acinar tissue."

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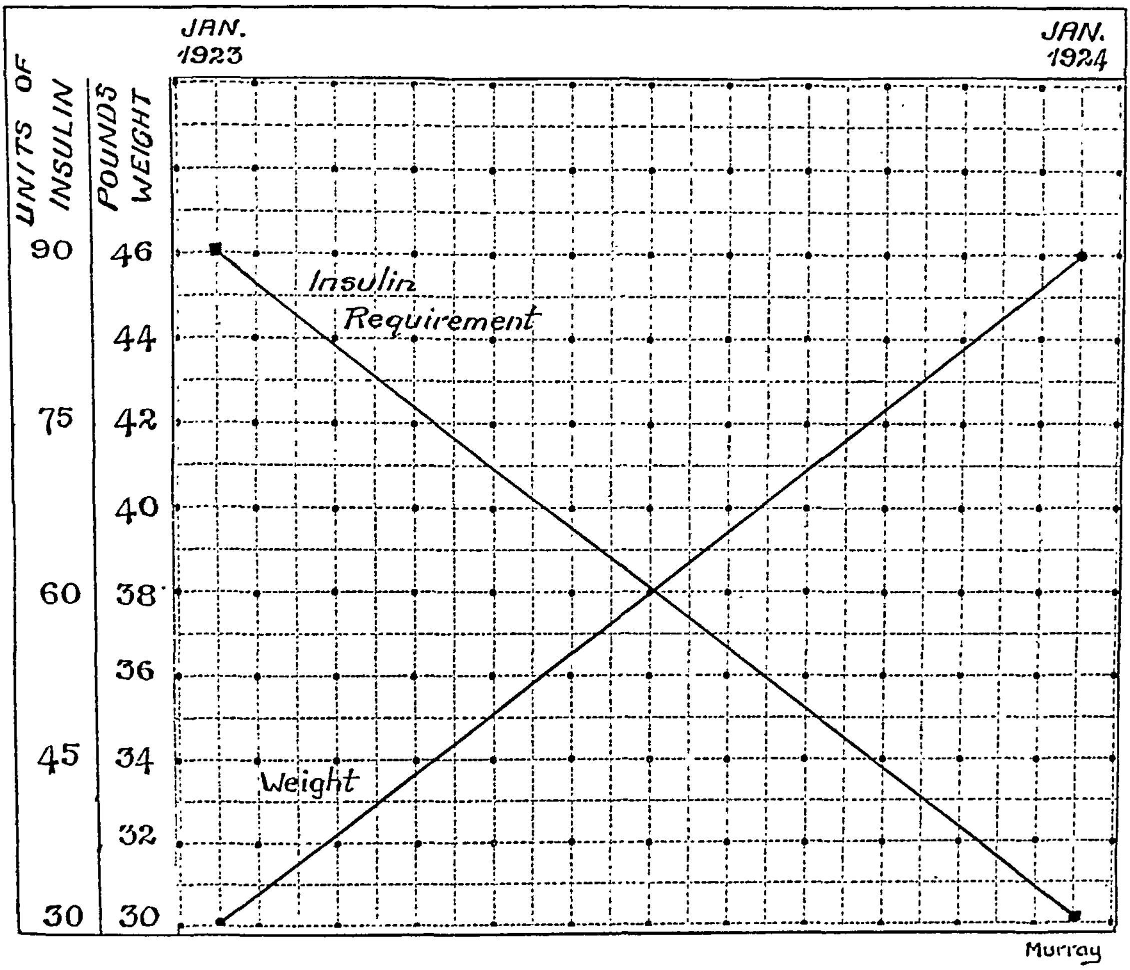
DESCRIPTION OF PLATE XXII

Chart showing decrease in insulin requirement and gain in weight of case reported.

Photographs of B. N. in January and in June, 1923.

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THE HISTOPATHOLOGY OF KALA-AZAR IN THE HAMSTER, MONKEY, AND MAN *

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The introduction of the hamster *Cricetulus griseus*, M.-Edw. as an experimental animal in the study of kala-azar in North China has furnished an abundance of material for histological research. This study has not only demonstrated clearly the pathological picture already described by many investigators in other animals and in man, but it has brought out some fundamental points in the pathology which have not been emphasized heretofore. I venture, therefore, to present our microscopic findings in the hamster in considerable detail and to compare them with our findings in the monkey and in man.

HISTORICAL

The first careful description of the histopathology of kala-azar was made by Christophers.[1] His accurate descriptions and interpretation of the pathological process have not been greatly added to by subsequent writers. He saw that the tissue changes which were due directly to the Leishman-donovan body involved mainly the presence and multiplication of the parasite in a certain group of cells represented by the endothelial cells of the liver and spleen capillaries and by the large mononuclear phagocytes of the blood and tissues. He recognized also the very important fact that Leishman-donovan bodies apparently proliferated within the cytoplasm of these phagocytic cells and were not destroyed by them, but ultimately caused their bursting or disintegration, followed by the liberation of the parasites into the tissues or blood stream. Christophers also appreciated the relationship between the pathological process in kala-azar and that in cutaneous leishmaniasis, which had been described by Wright [2] a short time before.

Since the appearance of these papers a large number of articles have appeared dealing with the pathology of the cutaneous and

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visceral leishmaniases. The British workers in India, and the Italian and French in the Mediterranean field have contributed most to the literature. More recently the South American cutaneous lesions have been carefully studied. Jemma and Di Cristina [3] describe the phagocytic cells as they appear in infantile kala-azar in Italy, designating them "blackberry-shaped cells" when they are distended with Leishman-donovan bodies. They discuss the distribution of the parasites and of the phagocytic cells and emphasize the relation of the latter to the vascular endothelium.

With the discovery of spontaneous leishmaniasis in dogs in Italy, and with the successful transmission of kala-azar to monkeys, dogs, and mice, an abundance of histological material became available. The best summary of the findings in experimental animals and in human cases is that of Laveran.[4] He shows that, in practically all positive cases, the liver, spleen, and bone marrow are involved in the pathological process, and that the intensity and duration of the infection determine to what extent other organs are involved. After the blood-forming organs, the lymph nodes and lymphatic tissue in other organs next become involved, then the organs whose loose areolar tissue normally contains many small blood vessels and wandering cells. Thus the intestines, testes, lungs, adrenals, pancreas, skin, kidneys, and meninges are likely to become involved in about the order named. The thymus has also been found to contain organisms. In all the visceral forms of the disease, however, including Indian, Mediterranean, canine, and experimental, the essential lesion appears to be the same. Emphasis has been laid by all authors upon the parasitizing of endothelial cells and the accumulation of these cells in favorable locations, particularly in the organs of the hematopoietic system.

Two recent papers on the histopathology of kala-azar deserve special mention. The first is that of Perry,[5] who describes his findings in the jejunum of two human cases of Indian kala-azar. The stroma of the mucosa was distended by extensive proliferation of the endothelial cells lining the lymph channels, and these cells were filled with Leishman-donovan bodies. The epithelium covering the villi was absent, but it persisted in the bases of the crypts between the villi. Although this, we believe, must have been due to post mortem change or to trauma in washing the tissues at necropsy, the findings emphasize the massive accumulation of parasitized cells

in this location. Interpreting the loss of epithelium in his sections as part of the pathological process, Perry emphasizes the possibility of the elimination of the parasite in viable form by way of the intestinal tract.

The second recent paper of interest is that of Shortt,[6] who describes the histological findings in two monkeys infected by intraperitoneal inoculation of liver and spleen emulsion from a case of Indian kala-azar. Shortt emphasizes the localization of the parasites in the endothelial cells of blood capillaries in the liver, spleen, and bone marrow, and in the interstitial cells of Leydig in the testis. In connection with the last of these findings, we shall describe later in this paper the findings in a human testis in which we are able to differentiate between the cells of Leydig and those containing parasites.

The invasion of the parenchymatous cells of the liver and suprarenal gland by Leishman-donovan bodies has been described by a few authors. Havet,[7] working in Laveran's laboratory, described the tissues of a dog infected experimentally by material from another naturally infected dog. In the liver he found the parenchymatous cells of the organ more heavily invaded by parasites than the capillary endothelium. Some of the liver cells also contained polymorphonuclear leucocytes. Such extensive invasion of the liver cells has not been reported by any other writer, but several have reported the occasional parasitization of liver cells, and we are able to corroborate this finding, as will be seen later.

Jemma and Di Cristina [3] show a colored drawing of a glandular cell of the suprarenal cortex invaded by Leishmania. We also found these cells parasitized in our most heavily infected hamster.

Nowhere in the available literature have we found a description of such focal lesions in the organs of either human cases or animals, as appear in the tissues which we are to describe in this paper. The appearance of a local proliferation of phagocytic cells forming masses of tissue with an epithelium-like appearance in many of the organs involved has been so striking as to command special attention, and the fact that the cells making up these masses often contain very few or no Leishman-donovan bodies indicates, we believe, a specific tissue response to the invading organism, which has not hitherto been emphasized.

Although no previous descriptions of the histopathology of kala-

azar in North China have been published, we have no grounds for believing that the disease or its pathology is different from that in India. The literature, furthermore, emphasizes the similarity in the pathology of the Indian and Mediterranean forms of the disease, and the similarity of findings in human cases and in experimentally and naturally infected animals.

Material for Study

The material used in the present study includes tissues from nineteen hamsters, two monkeys, and two human cases. Tissues from the hamsters were kindly provided by Dr. C. W. Young and Dr. H. J. Smyly of this department. The tissues were taken immediately after death, the animal either being sacrificed for the purpose or having been killed accidentally by liver puncture. The two monkeys were killed when moribund. The human tissues were secured one-half an hour and eighteen hours, respectively, after death. All tissues except those used for fat stain were fixed in Zenker's solution, were imbedded in paraffin, and cut at about five micra. Harris's hematoxylin counterstained with eosin was found to give a better combined tissue and parasite picture than the eosin-azur of Giemsa, and was therefore used throughout the study. Sections to show fat were fixed in formalin, cut by a freezing microtome, stained with Scharlach-R and counterstained with Harris' hematoxylin. For demonstration of the fibrous reticulum of the spleen, Mallory's aniline blue stain with carmine counterstain was used on Zenker-fixed paraffin sections.

Kala-Azar in the Hamster

The hamster, *Cricetulus griseus*, M.-Edw., is a grayish-brown field mouse with a white throat and belly (Fig. 1). The adult measures about 8 cm. in length and weighs about 30 grams, the male being somewhat larger than the female. The male has very large and prominent testes which cause the scrotum to protude backward from the body at the base of the short tail. These rodents inhabit the dry fields about Peking and are caught by the farmers in large numbers. They are easily kept in captivity, but do not breed unless furnished with a tunnel of earth in which to nest. They can be readily infected with kala-azar by the intraperitoneal inoculation of small amounts of an emulsion of infected human or animal tissue.

Infection by the use of cultures of *Leishmania donovani* has not yet been attempted. The two unique features of the infection in these animals are, first, the speed and ease with which a successful inoculation can be demonstrated, and, second, the increasing intensity of the infection in an inoculated animal over a long period of time with only slight jeopardy to the health of the animal. Of the two hamsters in our series which were longest infected, one was killed while in apparently good health on the 455th day after infection, and the other on the 351st day because it developed an abscess in one of its cheek sacs. Our 90-day hamster was definitely ill when killed. Some of the other hamsters infected in our laboratories have died a natural death probably as a direct result of the kala-azar infection, but their tissues could not be used because of rapid putrefaction. Young, Smyly, and Brown [8] have shown that smears of the spleen of hamsters reveal Leishman-donovan bodies on the third day after infection, and of the liver and bone marrow on the fourth day. Liver puncture, which can usually be done without killing the animal, reveals organisms with almost as great accuracy.

The macroscopic examination at necropsy of hamsters infected with kala-azar usually reveals nothing pathological except an enlarged spleen. In infections of one hundred or more days' duration this reaches enormous proportions. The organ may extend across the mid-line in the lower abdomen, reaching the right iliac fossa. The normal weight of the hamster's spleen is 0.05 to 0.1 gm. (0.15 to 0.3 per cent of the body weight). In hamster S. 22, infected 455 days, the spleen weighed 1.0 gm. or 3.1 per cent of the body weight. The liver ordinarily shows no macroscopic pathology, but in this same hamster it was greatly enlarged, extending nearly half way from the costal margin to the pubis, and was pale brown in color with many irregular light-red hemorrhagic areas on its surface. Other organs in this hamster might have shown macroscopic lesions had the organs been larger. This will be evident from the microscopic findings.

Microscopic Findings in the Hamster

A. *The Fundamental Tissue Reaction.* Before describing in detail the microscopic findings in the various organs, we wish to lay emphasis upon the specific tissue reaction of the mammalian body to the invading organism. This occurs in all tissues where a more or

less loose connective tissue stroma exists in which cells of the specific reactive type normally occur. The cell involved is the large mononuclear phagocyte of mammalian tissues, which has been variously designated as clasmatocyte (Ranvier), histiocyte (Aschoff), endothelial leucocyte (Mallory), macrophage (Metchnikoff).*

This cell, which we shall hereafter designate as the clasmatocyte, exists primarily in the reticulum of the spleen, lymphatic tissue, and bone marrow, but is found also in small numbers in the reticular connective tissue of the intestinal mucosa, the testis, and the other glandular organs. The subcutaneous and other connective tissues of the body may also contain it. In the liver the endothelial cells lining the intralobular blood capillaries (the so-called Kupffer cells) seem to belong to this group. This relationship is made simple if we assume, as seems probable, that the clasmatocyte is originally derived from the endothelium of the blood and lymph vessels.

In hamsters heavily infected with kala-azar, we find, in the organs mentioned above, islands of clasmatocytes forming solid masses of tissue (Figs. 28, 29, and 30). Wherever the structure will allow it, the cell masses occupy the reticular connective tissue. They may impinge directly on blood or lymph vessels, and may even project as minute polyps into the lumina of such vessels. The cells are closely set together as though forming a definite structure, and frequently give the appearance of a mass of pavement epithelium. This may be designated "clasmatocyte tissue" in the same sense as we speak of "lymphoid tissue."

The individual cells vary in size and shape according to their location, but always have a considerable amount of cytoplasm which is finely granular and stains fairly deeply with eosin. At the periphery of the cell masses, long processes of cytoplasm may project out into the loose connective tissue. The nucleus, usually single but occasionally multiple, is oval, reniform, or horse-shoe shaped. It is vesicular, contains one or two spherical nucleoli paracentrally located and three or four other chromatin masses which are usually flattened against the nuclear membrane. There is a very fine chromatin network which radiates outward from the chromatin masses and from

* The recent work of Cunningham, Sabin, and Doan,[9] separating the phagocytic cells of the spleen into two distinct groups, raises the question whether both these types of cell[3] are equally phagocytic for *Leishmania donovani*. We hope to investigate this point in the near future.

the nucleolus where it is densest. By these features this cell may readily be distinguished from glandular epithelial cells, from the interstitial cells of Leydig in the testis, from lymphocytes and plasma cells, and from smooth muscle cells. It is difficult to distinguish in nuclear structure from reticular connective tissue cells and from swollen endothelial cells. Its relationship to both these may be very close.

In the solid masses of these cells one sometimes finds scattered cells of other types; lymphocytes and plasma cells are most common, occasionally polymorphonuclear leucocytes and red blood cells are found, and of course, reticular connective tissue cells. Rarely lymphocytes or red blood cells are found within the cytoplasm of the phagocytes. In definite patches of clasmatocyte tissue the most striking thing is the slight degree to which the cells are parasitized. Most of them contain a few or a moderate number of parasites, but some cells, particularly in the centers of the cell masses, contained none. At the periphery some may be crowded with parasites. Occasionally a few cells, even when not heavily parasitized, have nuclei of a degenerated appearance, either staining diffusely blue, or taking a faint reddish tinge. When heavily parasitized, the nucleus of the cell may be shrunken or missing, and the cytoplasm may contain vacuoles which often seem to be occupied by the parasites. In very heavily infected tissues small holes containing a few Leishman-donovan bodies may be the only remnant of cells which have burst as a result of the proliferation of the parasites.

We have not seen definite mitotic figures among these masses of cells, although they do occur in some organs in cells which seem to be clasmatocytes. This lack of mitotic figures is strange, for it is difficult to explain the local accumulation of these cells except that they multiply *in situ*. Furthermore, the local multiplication seems to precede extensive parasitization for we have found the cell masses in the spleen, liver, and lymph nodes of mice infected only eleven days. Such an early development of groups of specialized cells and their mild degree of parasitization emphasizes the fact that this is a proliferative tissue response to a definite stimulus. The picture is clearest in animals in which the infection has reached a moderate degree. In those which are most heavily infected the very extensive proliferation of clasmatocytes and their intense parasitization hide somewhat the typical picture.

B. *The Characteristics of Leishmania donovani in tissues.* The typical Leishman-donovan body as seen in histological sections stained with hematoxylin and eosin is an oval body measuring about two by three micra. It has a definite pink-staining outline, but the cytoplasm is usually very faintly pink or colorless. Near one end is the spherical macro-nucleus about one micron in diameter, which usually has an eccentric clear circular area within it. The minute rod-shaped micro-nucleus points readily away from near the macro-nucleus toward the other end of the organism. The cell outline of the organism often cannot be seen, particularly if the cytoplasm of the parasitized cell stains deeply. Frequently the micro-nucleus is also invisible, perhaps because it is hidden by the macro-nucleus. Obviously it is impossible to distinguish with certainty a single *Leishmania* in a tissue section unless it is characteristic in appearance, and this may depend merely on its position with reference to the observer's eye. This explains why it has been possible for Young, Smyly, and Brown to find *Leishmania* in smears made from the spleen, liver, and bone marrow of hamsters on the third or fourth day after infection, whereas, in the same series of animals, our microscopic sections failed to reveal them until the sixth day. This is also probably the reason why sections from human necropsies sometimes fail to show organisms, while smears from the same organs made at necropsy do show them.

C. *The development of the pathological process in the organs involved. — Liver.* Leishman-donovan bodies are first found in the six-day hamster, where a few single Kupffer cells contain small numbers of parasites. In the nine-day animal a few small groups of proliferated endothelial cells project into the lumina of capillaries. By the eleventh day, there are many small islands of proliferated endothelial cells in some of which one or more cells contain parasites. In the portal spaces are a few parasitized clasmatocytes. The proliferation and parasitization steadily increased in intensity. *In the 115-day animal one liver cell was seen which contained parasites in its cytoplasm. 125-day hamster:* Islands of parasitized endothelial cells still larger and more numerous. Liver cells about them compressed slightly. A few liver cells contain parasites, sometimes numerous, and have cytoplasm slightly fragmented. Some of the Kupffer cells from capillaries have proliferated so as to project into the lumina of central lobular veins (Fig. 2). In some of these veins many parasitized clasmatocytes are present free among the blood cells (Fig. 3). This is in contrast to the portal veins, where no parasitized cells are found in the lumina. In the portal spaces several layers of clasmatocytes surround the veins, ducts, and arteries (Fig. 4). *351-day hamster:* The periportal proliferation of clasmatocytes is less marked, but otherwise the process is much farther advanced. Many liver cells are parasitized and show profound changes (Fig. 6). Large masses of endothelial cells

occlude many of the capillaries and are crowded with parasites; many contain large vacuoles, and a few are in fragments (Fig. 5). Many of their nuclei are shrunken. A few mitoses are seen among these cells (Fig. 7). The portal spaces show a marked increase of lymphocytes and plasma cells, besides the numerous parasitized clasmatocytes. A few small groups of plasma cells are present in the intralobular capillaries. Clasmatocytes sometimes abut directly on the lumen of the portal vein and a few are free in the lumen. In the central veins are masses of parasitized cells protruding into the lumen, but their continuity with the endothelium of the vein is not distinct (Fig. 8). An occasional flat endothelial cell lining a central vein contains a few parasites. *455-day hamster:* Profound changes have taken place in the liver tissue (Fig. 9). The lobular architecture of the organ is indistinct. The tissue is very friable. The liver cells are nearly all smaller than normal and some are shrunken or compressed into thin strands of tissue, between widely dilated capillaries (Fig. 10). They show all degrees of degeneration and parasitization. From ten to one hundred per cent of the liver cells in various fields are parasitized. In the areas where most degeneration is present some liver cells contain large fat globules. In some areas practically no liver cells remain, the loose tissue being made up almost entirely of heavily parasitized endothelial cells which float like balloons in the wide blood spaces. These cells are larger than in any previous hamster, sometimes measuring fifty micra in the smaller diameter, and containing hundreds of Leishmaniae in a single section. They are not grouped in very large masses, fifteen cells in one mass being about the maximum for one section. Many have no nuclei and others are vacuolated or fragmented. The portal spaces contain very few cells and the interlobular bile ducts have shrunken epithelium and practically no lumina. The central veins are not remarkable except that some of their flat endothelial cells contain parasites.

Spleen. The nine-day hamster was the first in which parasitized clasmatocytes were found. In later animals one notes first a larger number of single clasmatocytes in the pulp (11-day hamster), then a number of islands of clasmatocytes, most of which contained parasitized cells (15-day hamster). In the 90-day animal much of the pulp is replaced by clasmatocyte tissue, and some of the lymph follicles are invaded by it. *125-day hamster:* The picture is similar but more advanced, the pulp being more completely occupied by clasmatocytes, which are nearly all heavily parasitized, greatly swollen and often vacuolated (Fig. 11). Some have become disintegrated, leaving open spaces containing a few parasites. Many contain red blood cells as well as parasites. The blood capillaries and sinuses are somewhat compressed by the surrounding cells. The lymph follicles are more extensively invaded by clasmatocytes; some are almost completely replaced by clasmatocyte tissue (Fig. 12). *455-day hamster:* Huge heavily parasitized clasmatocytes dominate the picture. They occupy practically the entire pulp, and partly replace the lymph follicles (Figs. 13 and 14). The blood is mostly confined to definite vessels of narrow calibre. Most of these vessels have only a single layer of flat cells separating the lumina from the clasmatocytes, and even they are sometimes not visible (Fig. 15). Some vessels contain parasitized cells. Only thin trabeculae of fibrous tissue are present and seem to be compressed by the surrounding cells. The arteries of the lymph follicles often cannot be found. No mitotic figures are seen either in the pulp or in the splenic corpuscles where they are usually numerous.

Lymph Nodes. The first change seen was (15-day hamster) proliferation of

clasmatocytes just outside the mesial wall of the peripheral lymph sinus and beginning to invade the lymph follicles. A few of these cells contained Leishman-donovan bodies. This invasion of the follicular tissue steadily advanced, and the follicular architecture of the nodes was soon lost. The clasmatocyte tissue seemed to extend inward from the region of peripheral lymph sinus (Fig. 16). Smaller islands of this tissue were also present in the medullary portion of the node. *The degree of parasitization of any one group of cells seems to be in inverse proportion to the number of cells in the group.* In the 125-day hamster a considerable number of the parasitized clasmatocytes have several nuclei, sometimes grouped in the center, sometimes forming a ring (Fig. 17). This may be one method of division of these cells into a tissue-like group. *455-day hamster:* About half the lymphoid tissue is replaced by clasmatocyte tissue, and besides this many single clasmatocytes are present among the remaining lymphocytes. Nearly all the clasmatocytes are heavily parasitized, many are vacuolated or have burst. Lymph sinuses are dilated and contain many parasitized cells.

Bone Marrow. We failed to find Leishman-donovan bodies in sections of bone marrow until the twenty-fourth day after infection. This is in contrast to the finding of organisms in bone marrow smears in similar hamsters by Young, Smyly, and Brown on the fourth day after infection. It is difficult to obtain perfect sections of bones without decalcification, and that process frequently makes good staining impossible. This, together with the difficulty of recognizing single Leishman-donovan bodies in sections, probably accounts for the discrepancy. The bone marrow of the shafts of the femur and humerus is normally hematopoietic in the hamster, but very few clasmatocytes are found there normally. The femur, humerus, and vertebrae were the bones used in our study. *24-day hamster:* Active hematopoietic tissue lies between numerous fat cells. A few single clasmatocytes are present, crowded with Leishmaniae. *35-day hamster:* Throughout the marrow are many heavily parasitized clasmatocytes. They occur both singly and in islands of as many as twenty cells each. In the islands the cells fit close together like a pavement. *125-day hamster:* About one-third of the marrow is occupied by clasmatocytes, most of which are heavily parasitized, resulting in huge globular cells (Fig. 18). These cells often contain remains of other cells also, either red blood cells, lymphocytes, or granular leucocytes. *455-day hamster:* The clasmatocyte tissue comprises about one-half of the marrow (Fig. 19). The parasitized cells are huge and lie closely packed together. The hematopoietic cells lie in small compact groups between the masses of clasmatocytes. Erythropoiesis seems to be greatly reduced, and the blood vessels are apparently compressed.

Adrenal. Parasitized clasmatocytes were first seen in the 90-day hamster, in the interstitial tissue of the cortex and medulla. These increase in number and become grouped into small masses in later animals. In the 455-day hamster a few of the parenchymatous cells of the cortex contain Leishman-donovan bodies (Fig. 20). The parasitized cells show shrinking or fragmentation of the nuclei and fragmentation of the cytoplasm.

Outside of the five organs so far described, the localization of parasitized clasmatocytes is limited, except for rare instances, to the connective tissue of the body. The most favorable sites for this localization are the regions where loose areolar tissue is associated with good vascularity. Both of these factors probably operate to favor this localization as illustrated on the one hand by fat tissue, which is loose but not very vascular, and on the other hand by the

interstitial tissue of the pancreas and salivary glands, which is not loose but is very vascular. Where both conditions are present, as in the stroma of the intestinal mucosa, the pathological picture becomes very intense. In the kidney, however, the interstitial tissue contains parasitized cells only in the most intense general infections, and in the brain not at all. In order to conserve space the further findings will be described only from the 455-day hamster.

Fat Tissue. Wherever fat appears, single clasmatocytes, heavily parasitized, are scattered through it (Fig. 21). Very seldom are these accumulated into cell groups.

Gastro-Intestinal Tract. Stomach — heavily parasitized clasmatocytes are present in large numbers in the submucosa. They are also scattered singly throughout the stroma of the mucosa. *Duodenum* — the villi are mostly bulbous in shape due to the massing in the stroma of huge parasitized clasmatocytes (Fig. 22). These cells are packed closely together and replace the normal cellular content of this tissue. This is so especially toward the tips of villi, their bases often being relatively normal. The epithelium covering the villi is usually intact and no Leishmaniae are seen in the epithelial cells. The epithelium is separated from its basement membrane by a space filled with granular, pink staining material. This has been seen also in sections from other hamsters where no parasitized cells were present, and is probably a normal condition. Numerous parasitized cells are present also in the submucosa, except where Brünner's glands occur. *Jejunum* — much the same picture, but less intense. The tips of some villi show autolysis. *Ileum* — still less intense, perhaps because villi are small. Where lymph nodules occur they are partly replaced by parasitized "clasmatocyte tissue." Epithelium everywhere intact. *Cecum and Colon* — much less intense. Only single parasitized cells are present, scattered thickly through the mucosa and submucosa. Epithelium intact. In all sections from the gastro-intestinal tract, there are many single parasitized cells in the connective tissue septa of the muscularis and in the serosa.

Lungs. Both in the interlobular tissue and in alveolar walls parasitized cells are numerous. In the alveolar walls their relation to capillaries and epithelium is impossible to demonstrate. There is no apparent proliferation of capillary endothelium.

Heart. Throughout the myocardium and the sub-endocardial tissue heavily parasitized clasmatocytes are present in the interstitial tissue. Several groups of these cells are apparently associated with lymph vessels. Their relation to blood vessels is not so close. No parasitized cells are found lining the epi- or endocardium. The epicardial fat is heavily infiltrated with parasitized cells single and in groups. Muscle cells normal.

Pancreas and Salivary Glands. The interstitial tissue in these glands contains many single parasitized clasmatocytes.

Kidney. The kidney tissue in all previous hamsters was normal, but in this one a remarkable pathological condition is present. Throughout the cortex the proximal convoluted tubules are in all stages of degeneration, some normal, some represented only by a wrinkled basement membrane. Although there is very little formation of hyaline droplets in the tubular epithelium, the cells show all degrees of fragmentation and atrophy, the nuclei being the last portion of the cells to disappear. Many tubules contain hyaline casts. In proportion to the degree of tubular degeneration the interstitial tissue is infiltrated with clasmatocytes heavily laden with Leishmaniae. Several large and small areas

of this atrophy and infiltration are present and irregular strands of such tissue run through the cortex. The glomeruli are normal except for occasional distention of Bowman's capsule and for the presence of parasitized cells. These average one to a glomerulus in a section. They always appear to be filling the lumen of a capillary, but whether the cells are capillary endothelial cells or clasmatocytes plugging the vessels as emboli cannot be determined. In the medulla many collecting tubules are dilated and others are plugged with casts. In striking contrast to the cortex, the medulla contains no parasitized cells in its interstitial tissue.

Bladder, Testis, Epididymis, Seminal Vesicles. A few parasitized clasmatocytes are present in the connective tissue.

Prostate. Beneath the simple columnar epithelium is a densely cellular connective tissue. The invasion of this tissue by parasitized clasmatocytes approaches that of the intestinal mucosa in intensity (Fig. 23).

Brain and Meninges. Neither in the brain tissue itself nor in the walls of intracerebral blood vessels are any parasitized cells found. In the meninges and choroid plexus, however, they are rather numerous, usually in the loose tissue just outside blood vessels, but occasionally in the intimal cells of capillaries.

Eye. Parasitized cells found only in the loose outer layers of the posterior sclera where it joins the orbital connective tissue.

Skin and Cheek Sac. Beneath the epithelium in the loose areolar tissue parasitized clasmatocytes are very numerous.

MONKEYS

Two female monkeys, *Pithecus irus* (Syn. Macacus irus), were inoculated intraperitoneally with a suspension of liver and spleen from hamsters infected with kala-azar. The material for inoculation was ground up in a Rosenow tissue macerator and suspended in 10 c.c. of Locke's solution. Liver puncture first positive for *Leishmania donovani* on the 10th and 7th days respectively after inoculation. Both monkeys showed gradual progressive emaciation and lassitude, huddling up in one corner of cage. They became moribund on 45th and 48th days respectively and were killed by ether on those days.

Monkey 1. Necropsy. Very little subcutaneous fat. Spleen weighed 23 grams and was much enlarged, dark red in color, firm, not friable. Cut surface dark red, lymph follicles scarcely visible. Liver also much enlarged, weight 92 grams, otherwise grossly normal. Stomach contained an ulcer near pylorus 1 cm. in diameter, with raised margin and depressed center. Smear from this showed no parasites of any kind. Another ulcer was found in the rectum, probably due to taking temperature. Lymph nodes all slightly enlarged. Bone marrow of shaft of femur red and gelatinous. Other organs macroscopically normal.

Microscopic Sections.—Liver. Intralobular blood capillaries irregularly distended by intense proliferation of endothelial cells which often occlude the lumen entirely. In the endothelial cells are varying numbers of Leishmaniae, but rarely more than ten in a single cell in one section. Some cells contain none although they form part of a mass of endothelial cells. Many of the parasitized cells are vacuolated and Scharlach-R stain shows them to be heavily laden with fat. Mitotic figures are common in the masses of endothelial cells. Many nucleated red blood cells in the capillaries. Liver cells appear normal. None is

seen to contain Leishmaniae. The proliferation of endothelial cells extends on the one hand into the portal veins, the lumina of many of which are nearly occluded, and on the other hand into the central efferent veins, in which masses of parasitized endothelial cells lie in more or less intimate contact with the vessel wall. Blood-vessels contain very little blood. Portal spaces show little or no cellular infiltration and contain very few parasitized cells.

Spleen. Contrary to expectations only a very few Leishman-donovan bodies are found. Lymph follicles small and often partly replaced either by loose reticular tissue containing open spaces partly filled with blood, or by degenerating cellular material. This consists mostly of cells with large pale vesicular nuclei much like clasmatocytes, but the nuclei are often shrunken and the cytoplasm is indefinite in outline. There is considerable nuclear débris. Pulp consists mainly of this same semi-degenerated tissue, in which considerable blood is present. Considerable proliferation of fibroblasts, with an occasional mitosis. An occasional faintly-staining Leishman-donovan body is seen in the cytoplasm of a clasmatocyte, but the tissue pathology is far out of proportion to the apparent number of parasites.

Lymph Nodes. The picture varies in different nodes. In some the lymph nodules are well preserved, usually without germinal centers, and the invasion of clasmatocytes into lymph nodules is slight. In these nodes, however, Leishman-donovan bodies are present in most of the clasmatocytes, both in the reticulum of the node and in sinuses. In other nodes the lymphoid tissue is largely replaced by masses of cells with the characteristics of clasmatocytes, but there is much cellular fragmentation with free chromatin masses. A few Leishman-donovan bodies are seen in the clasmatocytes of these degenerated areas, but they usually stain faintly, and the impression received is that many more are present which have lost their staining power. Normal lymphoid tissue and degenerated regions may appear in the same section.

Bone Marrow. Very cellular, showing all the normal elements of active marrow, and in addition many clasmatocytes. These occur both singly and in groups. Most of the single ones are heavily parasitized. Where groups of cells occur, however, the cytoplasm of individual cells stains deeply pink, and only a few of the cells contain Leishmaniae. Most of these groups of cells have at the center a circular open space about 50 micra in diameter, about which a few flattened nuclei are usually present. Such spaces occur only in connection with these groups of clasmatocytes.

Adrenal. In the interstitial tissue between the glandular cells of the cortex are a few clasmatocytes containing Leishman-donovan bodies. The cortical cells contain practically no lipoid material. Otherwise the organ is normal.

Gastro-Intestinal Tract. In the stroma of the intestinal mucosa are varying numbers of parasitized clasmatocytes, most numerous in the jejunum. In the appendix, they are numerous about the periphery of a lymph follicle and in a lymph vessel leading away from this follicle (Fig. 24). Ulcers of stomach and rectum are not remarkable.

Lungs. The picture of endothelial proliferation with Leishman-donovan bodies is very similar to that in the liver. The lumina of the small arteries, veins, and capillaries are often practically occluded by masses of endothelial cells attached to the vessel wall (Fig. 25). Some of these cells contain Leishmaniae. The cells are often degenerated, containing large vacuoles. No cells of any kind are present in the alveoli, nor are any Leishman-donovan bodies

seen in cells lining the alveoli. There is marked congestion of those capillaries which are not occluded. In one section considerable interstitial hemorrhage is present. In the interlobular septa, cells heavily pigmented with carbon are present, but no Leishman-donovan bodies are found in those cells.

Heart. There are several patches in the myocardium where the muscle cells are swollen and hyalinized. There is one small hemorrhage. No Leishman-donovan bodies are found.

Kidney. A few swollen endothelial cells containing Leishmaniae are found in the veins and in capillaries of the glomeruli. Otherwise the organ is normal.

Brain. A few parasitized cells are found in the lumina of capillaries in the meninges and choroid plexus. Sections otherwise normal.

Sections of pancreas, salivary glands, urinary bladder, uterus, skin, and spinal cord all normal. The tongue and striated muscle in the neck show several colonies of sarcosporidia lying in or between muscle cells.

Monkey 2. Necropsy. Spleen and liver not so much enlarged as in Monkey 1, weighing 13 and 78 grams respectively.

Microscopic Sections. The picture of endothelial proliferation in the liver and lungs is even more marked than in Monkey 1 (Figs. 26 and 27). The other organs show in general the same histo-pathology. Leishman-donovan bodies are not well preserved in any organs except the colon and appendix. The impression is conveyed that extensive destruction of the parasites had taken place.

Human Cases

Case 1. P. U. M. C. Hospital No. 3853. Mrs. C. Y. T. G., a Chinese housewife, 38 years old, had had symptoms referable to kala-azar for nine months before death. She had had tartar emetic treatment in another hospital but had refused to continue it. She died of broncho-pneumonia on December 18, 1922.

Necropsy, by Dr. C. Bartlett (45 minutes post-mortem). Anatomical Diagnosis — Broncho-pneumonia, pleural adhesions, splenomegaly (485 grams) multiple old splenic infarcts, fatty liver, old and recent infarcts of right kidney, peritoneal and pericardial effusions.

Microscopic Sections. (H.E.M.) *Liver.* Extreme fat infiltration, most intense about the central veins. The few liver cells which are not laden with fat contain bile pigment, and a few are parasitized with *Leishmania*. In the blood capillaries the Kupffer cells are swollen and heavily parasitized, but there is very little proliferation of these cells into masses as in the hamsters and monkeys. Some of the parasitized cells also contain red blood cells or lymphocytes. In the walls and lumina of both portal and efferent veins are parasitized clasmatocytes. The portal spaces, however, show neither thickening nor cellular infiltration.

Spleen. Throughout the pulp, both in the reticulum and in the blood sinuses are myriads of heavily parasitized clasmatocytes. The infiltration is as heavy as in the 351-day hamster, but the parasitized cells are less swollen and fragmented, and there is less tendency to massing of parasitized cells into definite tissue. They encroach very little on the lymph follicles, which are about normal in size, although they have no germinal centers and often contain masses of hyaline material suggestive of amyloid. The pulp is greatly congested with blood, and also contains many lymphocytes and plasma cells. There is perhaps a slight increase of the fibrous reticulum.

Lymph Nodes. In some nodes the reticulum and sinuses contain diffusely

distributed clasmatocytes, most of which are heavily parasitized. In other nodes a large part of the reticulum is filled with definite "clasmatocyte tissue" as in the hamsters. When this occurs the clasmatocytes contain few or no Leishman-donovan bodies and their cytoplasm stains a deep pink. Carbon pigment, red blood cells and Leishman-donovan bodies are found together in some clasmatocytes. The lymph follicles are small and have no germinal centers.

Adrenal. Deep in the cortex are a few collections of lymphocytes, among which are a few parasitized clasmatocytes. Otherwise normal.

Lungs. Only an occasional parasitized clasmatocyte is seen in the alveolar walls. No endothelial proliferation is evident, as in the monkeys. There is a purulent bronchitis and in places a peri-bronchial pneumonia. There are also hemorrhages into groups of alveoli and in the patches of pneumonia. In nearly all the alveolar cavities are clasmatocytes, many of which contain carbon, but no Leishman-donovan bodies are seen in them.

Kidneys. A few parasitized clasmatocytes are found plugging glomerular capillaries.

Sections of heart, aorta, pancreas, gall bladder, urinary bladder, and ovary essentially normal. No specimens of bone marrow or gastro-intestinal tract were preserved.

CASE 2. P. U. M. C. Hospital No. 1757. H. S. T. Chinese man, student, 23 years old, was discovered to have kala-azar eighteen months before death. He failed to continue treatments, and died of a pneumococcus type III pneumonia on March 6, 1924.

Necropsy, by Dr. Y. K. Wang (18 hours post-mortem).

Anatomical Diagnosis. Lobar pneumonia, right; suppurative pluritis, left; suppurative pericarditis; bacteriemia, pneumococcus type III; splenomegaly (1,355 grams), enlargement of liver (12.5 cm. below costal margin); enlargement of mesenteric and cervical lymph nodes; hyperplasia of bone marrow.

Microscopic Sections. (H.E.M.) *Liver.* In the blood capillaries are many Kupffer cells heavily parasitized with *Leismania.* These are usually single, but a few groups of four or five cells are seen. In the portal spaces are many parasitized cells, often abutting directly upon lymph and blood vessels.

Spleen. Almost the entire tissue is composed of a reticulum containing "clasmatocyte tissue." Most of the clasmatocytes contain a few Leishmaniae; none is very heavily parasitized. The blood sinuses in general are widened and their walls thickened, but there is practically no proliferation of endothelial cells. There is a definite increase of the reticular fibrous tissue of the organ.

Lymph Nodes. The mesenteric nodes all show marked autolysis but in the loose reticulum which remains are many disintegrating cells heavily laden with Leishmaniae. The bronchial and aortic nodes show a loss of follicular architecture, although lymphocytes are still the predominating cells. There are also many clasmatocytes, both in the reticulum and in sinuses but only an occasional *Leishmania* is seen in them. There is no definite "clasmatocyte tissue." Several of the nodes contain areas of tuberculous caseation with giant cells.

Bone Marrow. (Rib.) This is perhaps better than the bone marrow of the femur for studying the specific reaction of kala-azar, since it is normally active marrow, and is therefore not complicated by the picture of hematopoiesis which is stimulated in the femur by this disease. This marrow contains no fat, and approximately eighty per cent of the space is occupied by clasmatocytes, packed

closely together into definite "clasmatocyte tissue." The picture is more striking than that found in any of the hamsters. Most of these cells contain a few Leishman-donovan bodies, some do not show them in the sections studied, none is very heavily parasitized. The normal hematopoiesis is almost entirely absent, especially the erythropoiesis. Most of the cells aside from the clasmatocytes seem to be lymphocytes. There are a few myelocytes and megakaryocytes. The vascularity of the marrow is greatly reduced.

Adrenal. In the medulla are a few collections of lymphocytes among which an occasional parasitized clasmatocyte occurs. In the fat tissue outside the organ is a focus of about one hundred cells, lymphocytes, plasma cells, and parasitized clasmatocytes.

Gastro-Intestinal Tract. There is marked post-mortem autolysis of the mucosa in most of the sections studied. *Stomach* — A few heavily parasitized clasmatocytes in superficial portion of stroma of mucosa. *Jejunum* — superficial portions of villi crowded with parasitized cells. *Ileum, cecum, appendix,* and *colon*—infiltration of parasitized cells less than in jejunum. Where lymph follicles occur, parasitized clasmatocytes are numerous in the neighboring submucosa.

Lungs. In the pneumonic areas the alveoli contain blood and leucocytes, among which are a few parasitized clasmatocytes. There is no endothelial proliferation.

Heart. In the myocardium are several areas in which the muscle cells are fragmented, and in these areas are collections of cells including polynuclear leucocytes, lymphocytes, plasma cells, and clasmatocytes. The latter are heavily laden with Leishman-donovan bodies. No endothelial proliferation or other abnormalities.

Kidneys. A few parasitized cells seen in lumina of capillaries in glomeruli and interstitial tissue. A number of convoluted tubules contain blood. Most of them are somewhat distended but only a few contain casts.

Testis. In the loose interstitial tissue are many single clasmatocytes more or less heavily parasitized (Fig. 31). The so-called "cells of Leydig" may be distinguished from clasmatocytes by the presence of more chromatin in the Leydig cell nuclei, the deeper staining of their cytoplasm, which is often of a bronze color, and the sharper cell outline. Since both our monkeys were females, we have had no opportunity to corroborate directly the statement of Shortt (6) that, in the monkey, the Leydig cells become parasitized with *Leishmania.* In the seminiferous tubules spermatogenesis is much decreased, only a few cells beyond the spermatogonia stage being present. Sections of prostate, urinary bladder, pancreas, and aorta are normal.

Discussion

The development of the tissue reaction in the hamsters is directly proportional to the duration of infection and to the multiplication of the parasites. Endothelial proliferation and the development of "clasmatocyte tissue" in the reticular connective tissue of favorable organs are the two main features of this reaction. The liver is the main site of the purely endothelial reaction; the bone marrow is the

best illustration of the interstitial reaction. Where both elements in the reaction may operate, as in the spleen, lymph nodes and stroma of the intestinal mucosa, the accumulation of parasitized cells is greatest. The remarkable tolerance of the hamster to an intense infection permits the accumulation of parasitized cells in practically every organ of the body where a reticular connective tissue is present. The only parenchymatous cells which become invaded, according to our observations, are those of the liver and the adrenal cortex.

In the two monkeys studied, although death was apparently imminent as a direct result of *Leishmania* infection, the tissue reaction was much more prominent than the parasitic invasion. We have considered the possibility that the organisms failed to stain in the spleen and lymph nodes as well as they did in those organs in the hamsters, but have discarded this possibility because of the satisfactory staining secured in the liver, lungs, and bone marrow of the monkeys. The destruction of both clasmatocytes and Leishman-donovan bodies in the spleen and lymph nodes may be related to the functions of those organs, and the products of this destruction may have been partly responsible for the rapid course of the disease in these animals. The remarkable amount of endothelial proliferation in the liver and lungs of the monkeys, practically occluding many capillaries in both organs as well as some of the veins in the lungs, may also have been an important factor in the progress of the disease. Although endothelial proliferation in the lungs was not very marked in the hamsters and was absent in the human cases, its intensity in the monkeys is in keeping with the well-known function of these cells to act as scavengers for carbon, blood pigment, and other foreign substances found in the parenchyma of those organs.

The two human cases here reported, although not completely studied, showed essentially the same lesions as the experimental animals. Specific treatment and terminal infections may have caused some alterations in the picture. It is well known that acute infections like pneumonia will cause remission of the symptoms of kala-azar, with reduction in the size of the spleen and inability to recover *Leishmania* from spleen puncture. Nevertheless, in the two cases reported "clasmatocyte tissue" was well developed in the spleen, in some lymph nodes, and in the bone marrow, and the endothelial reaction in the liver was typical. If tissues from the intestine and mesenteric lymph nodes in Case 2 could have been carefully secured

immediately after death, the picture produced in the hamsters would probably have been more accurately duplicated.

The histological picture produced in the cutaneous form of leishmaniasis is essentially the same as that in the visceral form of the disease as here described. Wright [2] stated in his original description of "tropical ulcer" that "these large cells, over extensive areas, are very numerous and constitute the principal part of the infiltration." The local skin lesion might therefore be termed a "clasmatocytoma," which becomes an ulcer only when the skin surface is broken, and a granuloma only when the ulcer is in the process of healing.

Lesions involving mainly the capillary endothelial cells and the clasmatocytes are not limited to kala-azar, but in no other disease is the intensity of the reaction nearly so great.* Typhoid fever probably comes nearest to kala-azar in this type of reaction. In fact the histological picture of that disease as described by Mallory [10] could almost be duplicated by the cellular reaction in the intestine, mesenteric lymph nodes, spleen, and bone marrow of a moderately severe case of kala-azar. The liver lesions in the two diseases also have much in common. There are two main differences in the lesions of the two diseases. The first is the presence of the protozoan parasites in the cells in kala-azar. The second is the fact that the typhoid lesions, being stimulated by a relatively toxic organism, usually go on to necrosis, whereas those of kala-azar in which the invading organism is relatively non-toxic for individual cells, are mainly productive in nature, and never show necrosis of more than individual cells.

The descriptions by Councilman and his co-workers,[11, 12] of the lesions produced by diphtheria in the spleen and lymph nodes, and by variola in the spleen, lymph nodes, and bone marrow also involve chiefly the clasmatocyte and are very similar to those seen in kala-azar.

* Since completing this paper, my attention has been called by Dr. S. T. Darling to his work on histoplasmosis.[13] The lesions caused by the parasite *Histoplasma capsulatum* are almost identical with those produced by *Leishmania*. The parasitization and proliferation of endothelial cells are similar to the lesions in kala-azar, and the tubercle-like nodules of histoplasmosis, occurring in the lungs and intestine, correspond closely to the localized lesions of cutaneous leishmaniasis. Since no more cases of the disease have been encountered since Darling's original report, it remains somewhat of a mystery. Darling at first considered the parasite *Histoplasma capsulatum* to be a protozoön, but he now agrees (personal communication) with da Rocha Lima [14] that it is probably a Cryptococcus, closely related to *Cryptococcus farciminosus*, which causes epizoötic lymphangitis in horses.

In the study of cells by observing their ability to take up vital dyes and harmless particles, such as carbon, the clasmatocyte has received a great deal of attention. Most of the work done with these substances consists of single or repeated injections of the material to be used as an indicator of cell activity, followed by histological study of the cells in sections or smears. Foot,[15] for instance, made histological studies of these cells in tissue sections after a series of intraperitoneal injections of trypan blue, intravenous injections of lampblack and subcutaneous injections of agar. Simpson [16] studied these cells in the circulating blood after repeated intravenous injections of various colloidal dyes and particulate suspensions. Kala-azar offers another method of studying these cells, and the present study has revealed the development of the cells involved into a fixed tissue which is probably one expression of the function of such cells. We are dealing with a parasite of very low virulence which selects the clasmatocyte, or is selected by it, and which multiplies within the cell but destroys it only by its numbers, not apparently by toxins. At the same time it stimulates the specific cell to local multiplication, thereby furnishing a factor not supplied by foreign bodies, such as carbon particles. It may be that further study of the disease in experimental animals will add more to our knowledge of the function of the clasmatocyte.

Summary

1. The hamster, *Cricetulus griseus*, is an ideal animal for the experimental study of kala-azar because of the ease with which it can be infected and because of its tolerance of an intense infection.

2. The specific tissue reaction to the infection consists of endothelial proliferation and the formation of solid masses of "clasmatocyte tissue." The liver, spleen, lymph nodes, and bone marrow are the chief sites of formation of this tissue. The formation of tissue masses often appears to be in advance of the parasitization of individual cells.

3. In the most advanced infections the parenchymatous cells of the liver and adrenal cortex become parasitized, and severe degeneration of the liver occurs. The spleen reaches huge proportions, lesions appear in the kidneys, and parasitized clasmatocytes appear in the connective tissue of practically all organs and tissues.

4. In advanced infections the stroma of the intestinal mucosa is

the site of massive accumulation of parasitized cells. Contrary to Perry's observations in human cases, the epithelium covering parasitized villi is intact, if post-mortem trauma and autolysis of the mucosa are avoided.

5. In two monkeys experimentally infected, endothelial proliferation dominated the pathological picture, leading to practical occlusion of capillaries and veins of the liver and lungs.

6. Two human cases show practically the same histological picture as that seen in experimental animals. Treatment by tartar emetic and the influence of terminal infections are probably responsible for the differences observed.

7. In a few other diseases, notably in typhoid fever, the clasmatocyte is the principal cell involved in the tissue reaction. In typhoid fever, however, the toxicity of the invading organism prevents the development of so productive a lesion as that seen in leishmaniasis.

8. The further study of tissues from animals experimentally infected with *Leishmania* may contribute to our knowledge of the origin, activities, and functions of the clasmatocyte.

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DESCRIPTION OF PLATES XXIII–XXVIII

Fig. 1. The hamster, *Cricetulus griseus.* About 2/3 life size.

Fig. 2. Liver of 125-day hamster. Endothelial cell containing Leishman-donovan bodies and projecting into lumen of central lobular vein. x 605.

Fig. 3. Liver of 125-day hamster. Parasitized clasmatocytes free in lumen of central lobular vein. x 512.

Fig. 4. Liver of 125-day hamster. Portal space crowded with parasitized clasmatocytes. x 251.

Fig. 5. Liver of 351-day hamster. Capillary occluded by a mass of parasitized endothelial cells. Adjacent liver cells also parasitized. x 745.

Fig. 6. Liver of 351-day hamster. Parasitized liver cell and mitoses in adjacent endothelial cells. x 1248.

Fig. 7. Liver of 351-day hamster. A group of endothelial cells, one heavily parasitized, another in mitosis. x 1248.

Fig. 8. Liver of 351-day hamster. Heavily parasitized endothelial cells in a central lobular vein. x 745.

Fig. 9. Liver of 455-day hamster, showing disintegration of liver tissue and huge parasitized endothelial cells. x 84.

Fig. 10. Liver of 455-day hamster. Liver cells compressed between huge parasitized endothelial cells. x 745.

Fig. 11. Spleen of 125-day hamster. Heavily parasitized cells in pulp and blood sinuses. x 745.

Fig. 12. Spleen of 125-day hamster, showing "clasmatocyte tissue" partly replacing a lymph follicle. x 130.

Fig. 13. Spleen of 455-day hamster, showing "clasmatocyte tissue" occupying most of the spleen substance. x 130.

Fig. 14. Spleen of 455-day hamster. Huge parasitized clasmatocytes in spleen pulp. Megakaryocyte, without Leishmania, stands out in contrast. x 745.

Fig. 15. Spleen of 455-day hamster. Parasitized cells partly lining blood sinus. One cell in sinus contains both red blood cells and a Leishman-donovan body. x 745.

Fig. 16. Lymph node of 90-day hamster. "Clasmatocyte tissue" invading lymph follicles from periphery of node. x 84.

Fig. 17. Lymph node of 125-day hamster. A multinucleated clasmatocyte containing many Leishman-donovan bodies. x 1248.

Fig. 18. Bone marrow of 125-day hamster. "Clasmatocyte tissue" at center of a small tongue of marrow in head of femur. x 233.

Fig. 19. Bone marrow of 455-day hamster. "Clasmatocyte tissue" comprises about one half of the marrow. x 84.

Fig. 20. Adrenal of 455-day hamster. Gland cell of cortex containing Leishman-donovan bodies. x 1864.

Fig. 21. Parasitized clasmatocytes in stroma of fat outside epididymis of 351-day hamster. x 1025.

Fig. 22. Duodenum of 455-day hamster. Villus, with stroma filled with parasitized clasmatocytes. x 419.

Fig. 23. Prostate of 455-day hamster. Submucosa filled with parasitized clasmatocytes. x 419.

Fig. 24. Appendix of Monkey 1. Lymph vessel in serosa containing parasitized clasmatocytes. x 810.

Fig. 25. Lung of Monkey 1. Capillary in alveolar wall, occluded by parasitized clasmatocytes. x 932.

Fig. 26. Liver of Monkey 2. Intense endothelial proliferation occluding a blood capillary. A number of lymphocytes and plasma cells are present among the endothelial cells. No typical Leishman-donovan bodies are found. x 419.

Fig. 27. Lung of Monkey 2. Interlobular vein entirely occluded by endothelial proliferation. x 251.

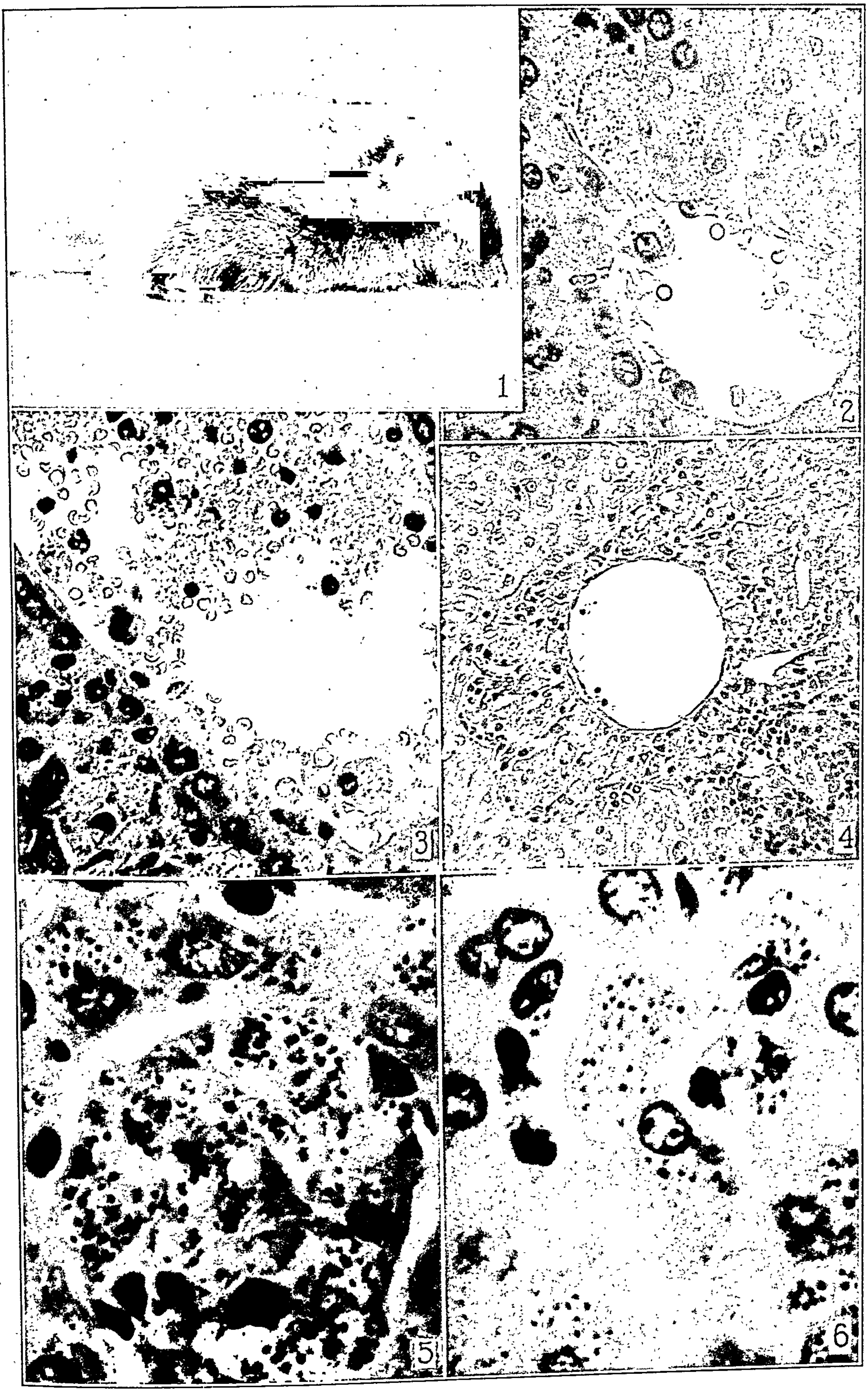
Fig. 28. Spleen of 138-day hamster. A mass of clasmatocyte tissue in which the cells toward the center contain very few Leishman-donovan bodies. x 783.

Fig. 29. Bone marrow of 110-day hamster. Some clasmatocytes contain no parasites. One contains both parasites and a red blood cell. x 1400.

Fig. 30. Lymph node of 351-day hamster. Clasmatocyte tissue partly replacing a lymph follicle. x 783.

Fig. 31. Human testis, Case 2. Parasitized clasmatocytes in the interstitial tissue. Note deep staining of nuclei and cytoplasm of cells of Leydig. x 783.

Figures 28 to 31 are colored camera lucida drawings. Tissue was stained with hematoxylin and eosin.



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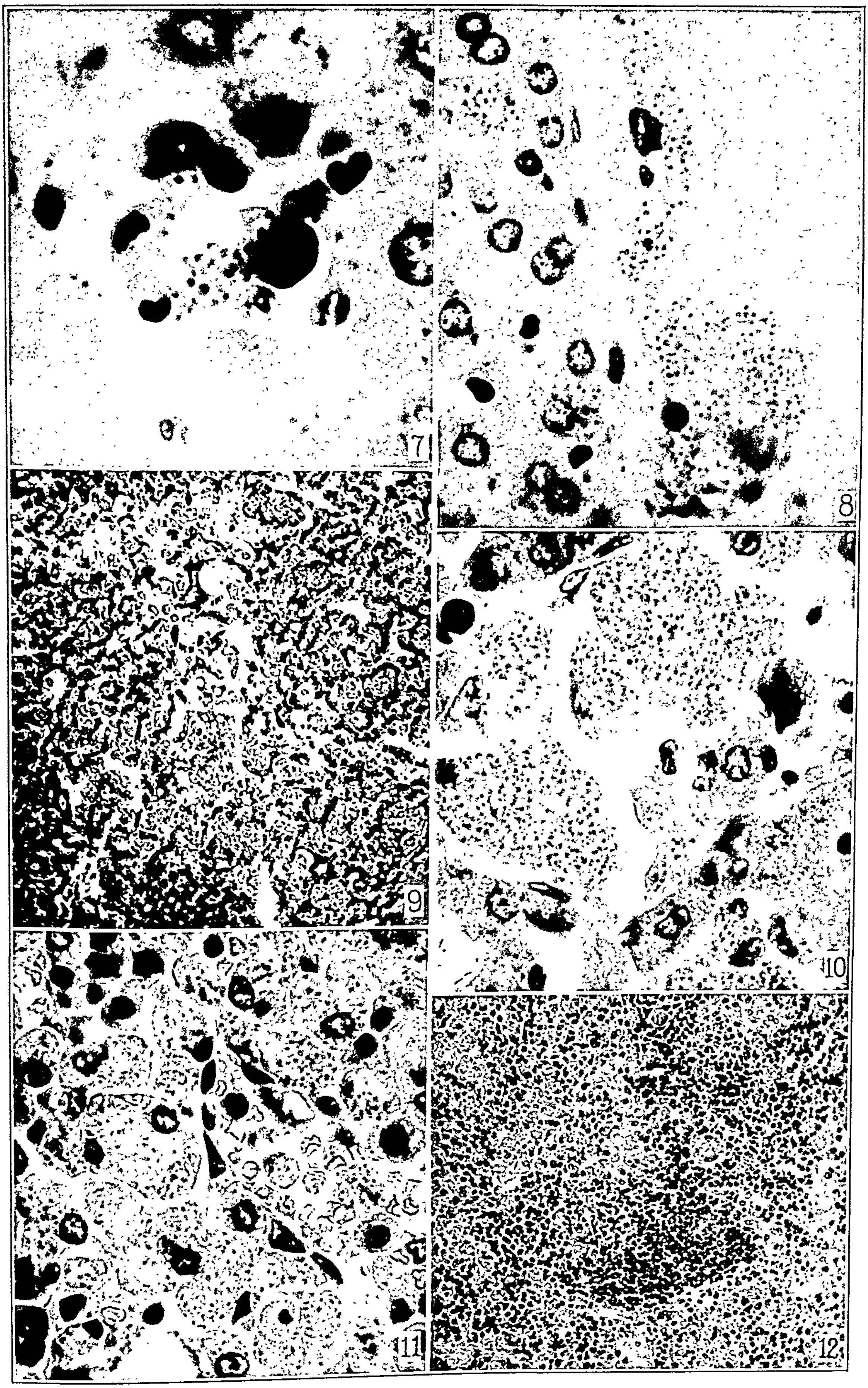

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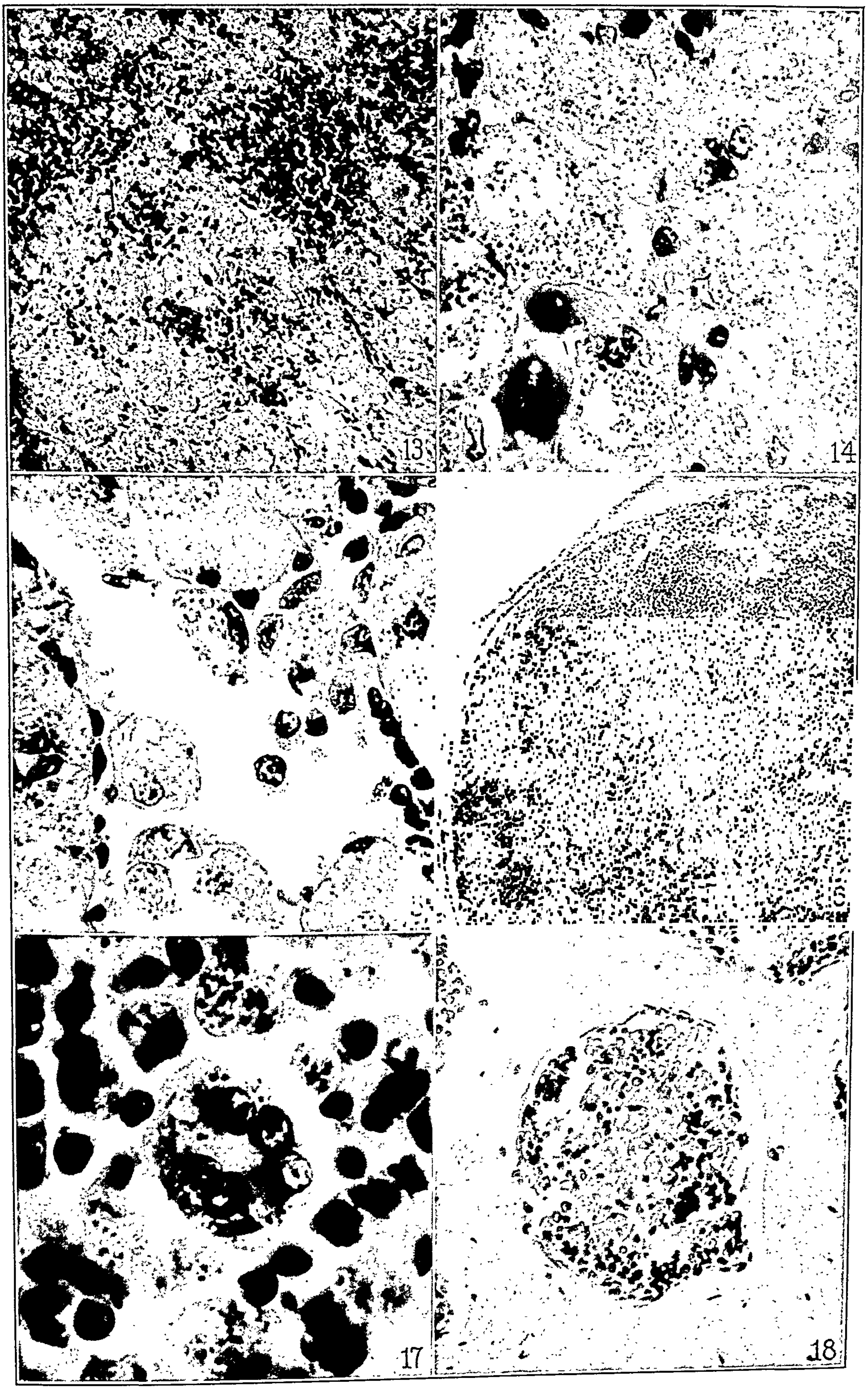

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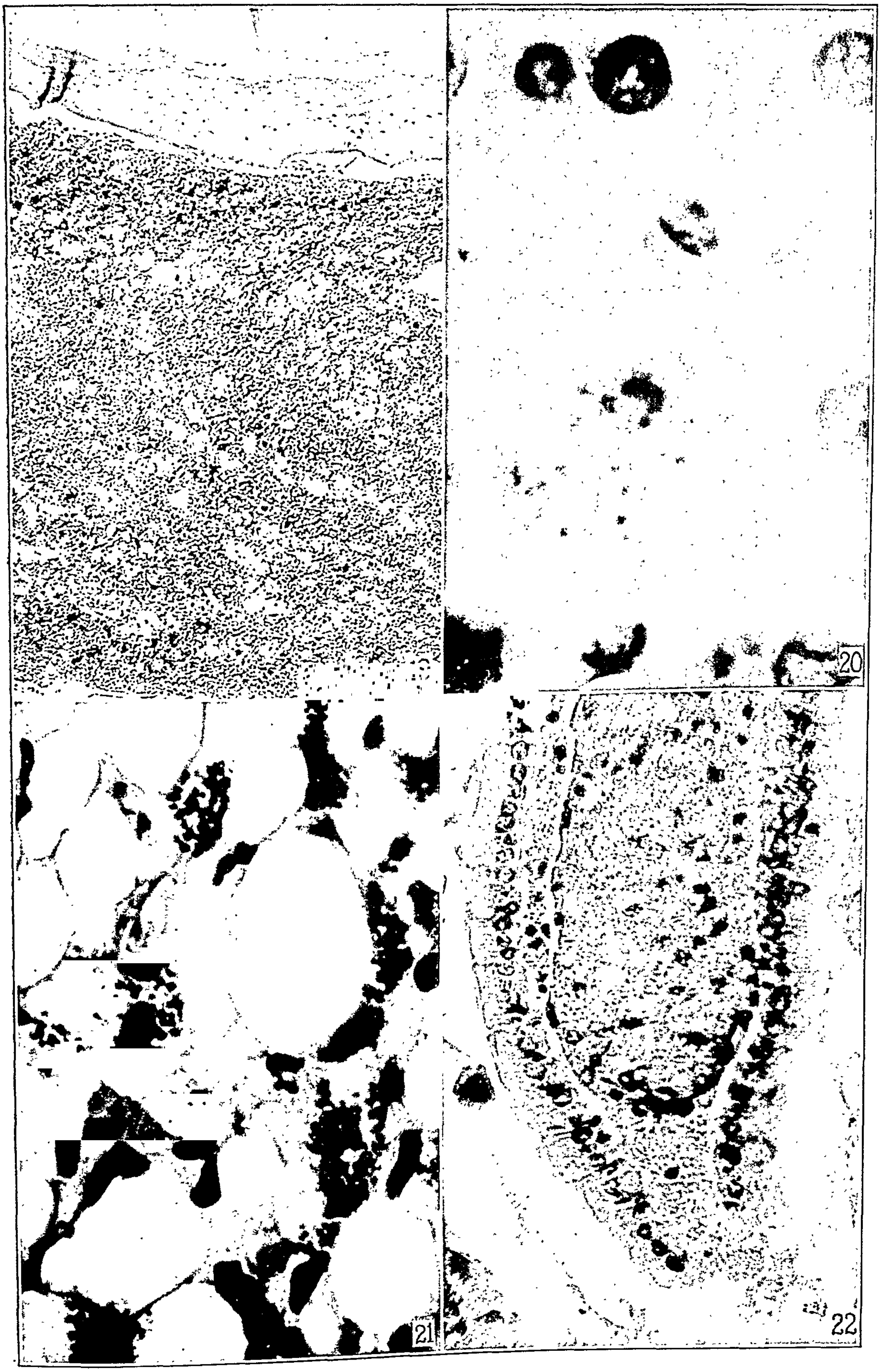

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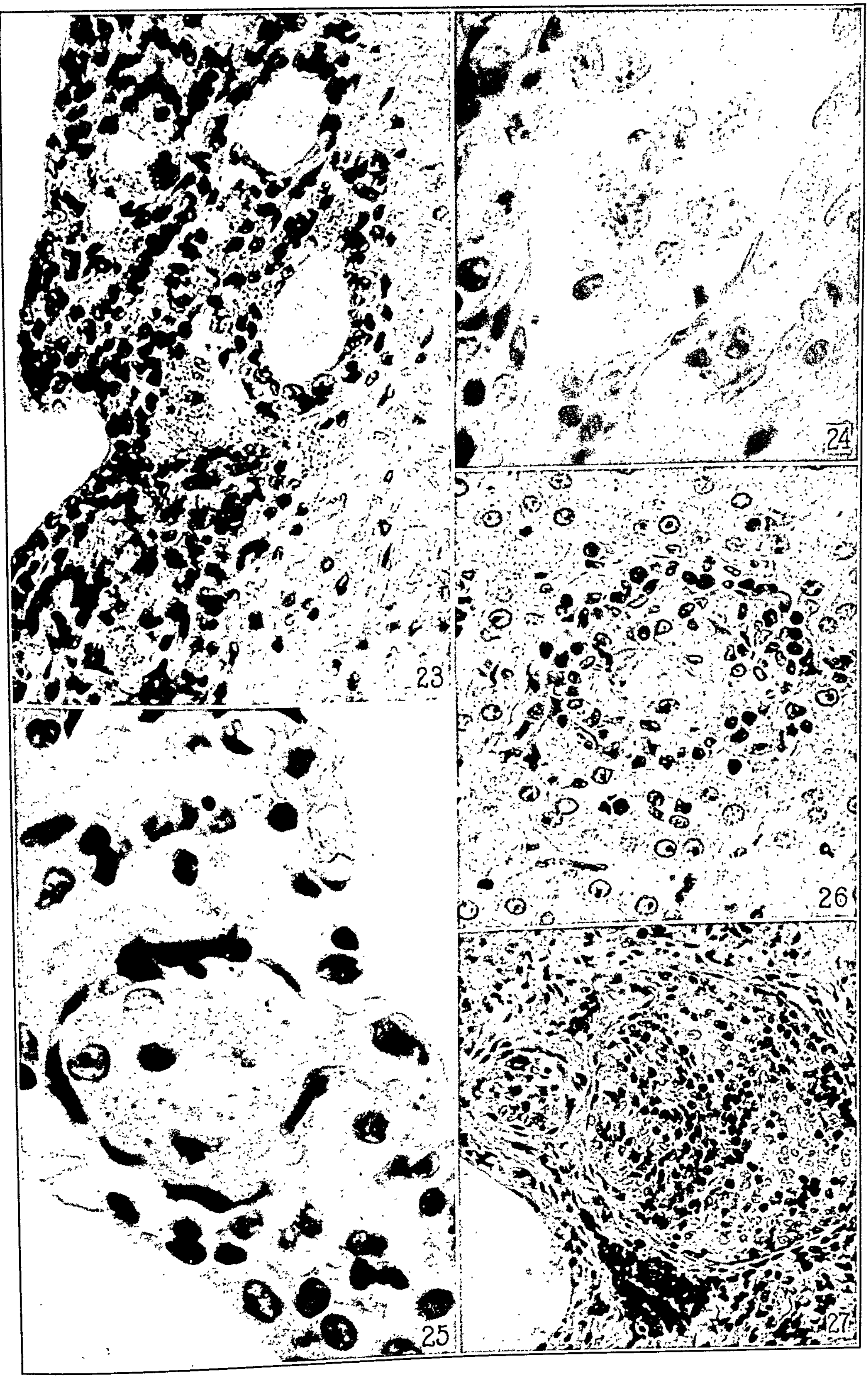

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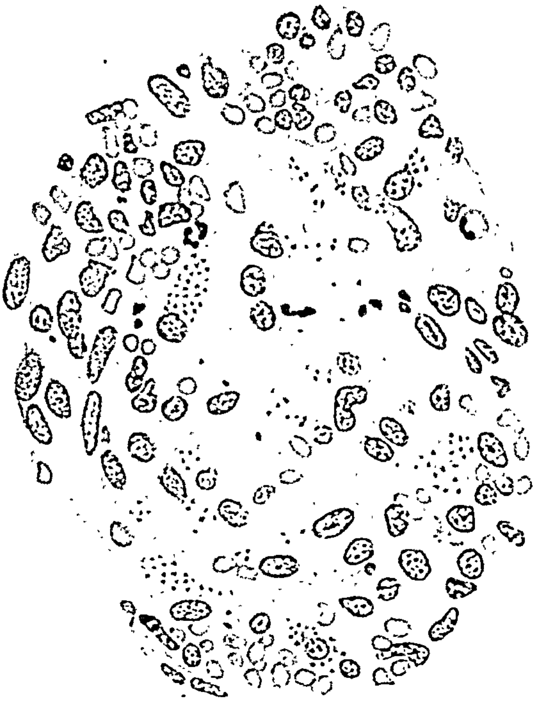

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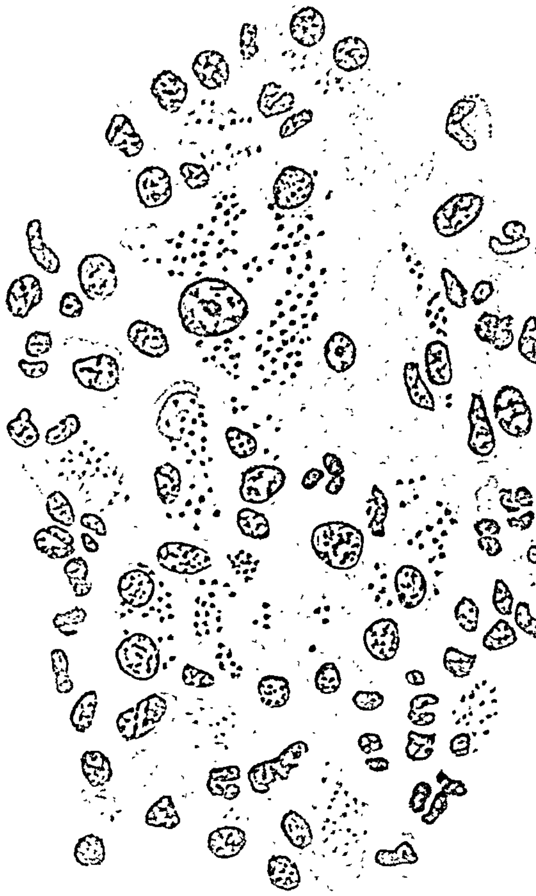

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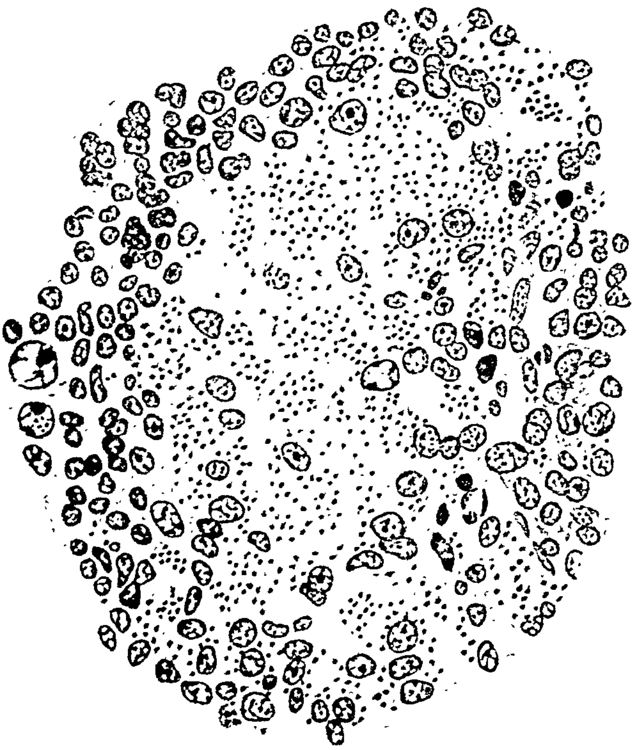

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PHAGOCYTOSIS OF ERYTHROCYTES IN THE BONE MARROW, WITH SPECIAL REFERENCE TO PERNICIOUS ANEMIA *

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The processes which bring about the destruction of red blood corpuscles have been studied intensively in man and in the lower animals by physiologists and pathologists, but our information regarding them still remains extraordinarily incomplete. The situation is presented clearly in the excellent review of the literature on the subject by Rous,[1] who discusses the various methods of blood destruction in different species and under normal and pathological conditions. Such a survey indicates the urgent need for more clinical observations and more animal experimentation directed towards the solution of the problem.

Among the processes which have been considered to account for the destruction of erythrocytes, that of phagocytosis by fixed tissue cells or by wandering cells has long been recognized, but, in spite of the fact that it has been frequently described as occurring under normal and pathological circumstances, it has not usually been regarded as a factor of particular significance in blood destruction in man. The recent publications of Aschoff [2] and Lepehne,[3] however, on the phagocytic cells of the so-called "reticulo-endothelial system" of the liver, spleen, lymph glands, hemolymph glands, and bone marrow, and their rôle in the pathogenesis of certain types of hemolytic jaundice, make it apparent that phagocytosis may, at least occasionally, become the predominant element in blood destruction. When one considers the relative bulk of the spleen and liver in comparison to that of the normal active bone marrow it is not surprising that the attention of investigators has been directed chiefly to these larger organs and that the bone marrow has received little consideration as a possible site of blood destruction. There are, however, pathological conditions in which the fatty marrow of the long bones becomes replaced by a cellular tissue and the bone marrow becomes an organ of material size. This happens, for instance, in

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pernicious anemia, and the fact that the hyperplastic bone marrow may be the seat of a striking amount of phagocytosis of red blood corpuscles has led us to a consideration of the part played by the bone marrow in blood destruction in this disease.

The occurrence of phagocytosis of red corpuscles in the bone marrow in pernicious anemia has been recognized ever since the earliest observations on the histology of the disease. Cohnheim [4] mentioned it in 1876, and in 1877 Osler and Gardner [5] described a case in which three or four cells, each containing five or six red corpuscles, were found in a single field of the vertebral bone marrow. Riess,[6] writing in 1881, was greatly impressed with the large numbers of phagocytic cells in the bone marrow in pernicious anemia, and raised the question of their relation to the pathogenesis of the disease. Dickson[7] says, "Pigment cells, and cells containing erythroblasts and red corpuscles are seen in very great numbers, for example, in pernicious anemia . . ." It is unnecessary to cite the extensive literature in which similar observations have been made, for the existence of phagocytosis in the bone marrow in pernicious anemia is commonly accepted. MacCallum [8] mentions it and illustrates it in his "Text Book of Pathology." With few exceptions, however, the writers have contented themselves with incidental references to the process and little attention has been paid to its possible relation to the pathogenesis of the disease. This aspect of the subject assumes some importance in the light of the current hypothesis, based largely on the abnormal increase of bilirubin in the blood serum and in the bile, that pernicious anemia is associated with an increased blood destruction. The failure to account for this excessive blood destruction satisfactorily in any other way raises the question as to whether the phagocytosis of erythrocytes, so frequently observed in the bone marrow, may be a factor in producing the destruction. The answer to this question depends in part, at least, on whether phagocytosis is more prominent in the bone marrow in pernicious anemia than in other diseases and on the constancy of its occurrence in pernicious anemia.

The phagocytic cells of the bone marrow arise either from the vascular endothelium or from the reticulum.[7] With the material and stains available it has not been considered possible to differentiate accurately between these two types of cell and no attempt has been made to do so. In all probability the majority of the phago-

cytes, and certainly those which are free, wandering cells, are to be classed as "endothelial leucocytes" in the nomenclature of Mallory [9] or as "clasmatocytes" in the nomenclature of Sabin.[10]

In this investigation a distinction has been made between the presence in a phagocyte of erythrocytes containing hemoglobin and the presence of granules of so-called "hemosiderin" in which the iron is in such a form as to give the Prussian blue reaction. This distinction seems to be justified because certain diseases, such as pernicious anemia, are characterized by a predominant phagocytosis of hemoglobin-containing cells, while others, such as hemochromatosis, are marked by the presence of large amounts of hemosiderin within the cells, and still others, such as cirrhosis of the liver, frequently show both features to a more or less similar degree. In many instances, special stains for iron have been made but the yellow-brown or yellow-green color of the latter pigment is so characteristic that they are not usually necessary. There can be no question that the hemosiderin is derived from hemoglobin but it is more difficult to determine whether this pigment has been formed within the phagocyte or whether it was already formed when taken up by the phagocyte. As far as it goes, the histological evidence indicates that in most cases certainly the bone marrow phagocyte ingests the red corpuscles and that the hemosiderin is formed from hemoglobin within the phagocyte. This is suggested by the presence of hemoglobin-containing cells and granules of hemosiderin in the same phagocyte and by the fact that phagocytes which contain erythrocytes of normal appearance may also contain masses, staining for iron, of the size and shape of a red corpuscle. These masses apparently represent the early phases in the transformation of hemoglobin into hemosiderin — perhaps before the complete dissolution of the stroma of the red corpuscle.

The relative extent to which hemosiderin granules or hemoglobin-containing red corpuscles are found in the phagocytes may merely indicate the capacity of the phagocytes for retaining or storing the ingested material, but it is also quite possible that it indicates the intensity or severity of the phagocytic process. The fact that the phagocytosis of hemoglobin-containing red cells is particularly prominent in certain diseases associated with anemia and jaundice would seem to make such an hypothesis quite plausible. If the pigment remains in the phagocyte for a certain length of time it is

probable that the change from hemoglobin to hemosiderin occurs. Rich [11] states that the splitting of hemoglobin into bile pigment and an iron-containing residue by phagocytic cells of mesodermal origin, in tissue cultures, can be watched under the microscope. Pearce,[12] reviewing his own work and that of Karsner, Amiral, and Bock,[13] on the production of phagocytosis of erythrocytes in the lymph nodes of splenectomized animals by the injection of hemolytic sera, says that the phagocytosis "reaches its height somewhere between twelve and twenty-four hours after injection, and then the destruction of corpuscles goes on, so that at forty-eight hours there is nothing left but pigment and corpuscular fragments. Furthermore, with the passage of time the individual phagocytes become more and more filled with erythrocytes until about twelve to twenty-four hours, at which time there is a disappearance of the erythrocytes with the substitution of the pigment granule." If this evidence can be accepted as throwing a general light on the situation in man, one is justified in assuming that phagocyted red corpuscles which contain unchanged hemoglobin have probably not been inside the phagocyte for more than twenty-four hours. Thus the association of a histological lesion showing extensive phagocytosis of hemoglobin-containing erythrocytes, together with an increase of the amount of bilirubin in the blood plasma would indicate an acute destructive process in which large numbers of erythrocytes have been ingested by phagocytes and have been again transferred, probably partially destroyed, into the blood stream before the change from hemoglobin to hemosiderin has had time to take place. On the other hand, the presence of a large amount of hemosiderin, with or without accompanying erythrocytes, in the phagocytic cells would suggest a much less acute phagocytic process, and the relative proportion of hemoglobin-containing erythrocytes to hemosiderin granules would give an indication of the rate of blood destruction and of pigment change.

Control Observations

In order to determine the frequency and extent to which phagocytosis of red blood corpuscles occurs in the bone marrow in conditions other than pernicious anemia, observations have been made on four specimens of normal vertebral bone marrow and on sections of bone marrow (mostly vertebral) from 130 autopsies of cases dying

in a general hospital. The latter were, with very few exceptions, unselected and represent the various types of disease which are usually seen in the Pathological Service of such an institution. The amount of material and the wide range of diagnosis is thus sufficient to give at least a general conception of the frequency and extent to which phagocytosis occurs in the bone marrow in man. Grohé,[14] in 1884, and Geelmuyden,[15] in 1886, reported similar studies on the bone marrow. Both of them observed phagocytosis in a variety of conditions, and neither felt that it was much more extensive in the few cases of pernicious anemia examined than in a number of other diseases.

All of the sections of the vertebral bone marrow from four normal adults, in whom death was the result of traumatism, showed the presence of intracellular hemosiderin granules. In two instances only occasional pigment granules were found; in the third there was a considerable number of phagocytes containing coarse pigment granules — an amount that under other circumstances might have been considered to be abnormally great. In only one specimen were any phagocyted erythrocytes seen, and in this instance they were extremely rare. They were apparently intact and contained unaltered hemoglobin. From these few observations one can merely conclude that phagocytosis of erythrocytes occurs normally in the human bone marrow, but that it occurs only to a very limited extent. In this connection it is worth noting that Van den Bergh[16] has found bilirubin to be present in the sera of many normal individuals and the suggestion may be made that the normal variations in bilirubin depend on the activity of the phagocytic cells of the bone marrow and of the other organs of the reticulo-endothelial system.

In the study of the specimens of bone marrow from 130 patients dying in the hospital from various causes, an attempt has been made to estimate the extent to which erythrocytes and hemosiderin granules are present in the phagocytic cells. It is, of course, appreciated that this cannot be done with any high degree of accuracy because quantitative methods are not applicable, but it has been satisfactory to find that observations on the same specimen at widely separated intervals of time have been essentially in agreement. Specimens from different parts of the bone marrow in the same case may vary somewhat in histological picture but the differ-

ence is not sufficient to militate against the general value of observations on one or two sections. It is felt that the observations reported may be taken as rough representations of the amount of phagocytosis present. No further accuracy is claimed for them, and no further accuracy is necessary for the purpose in view.

In almost every case phagocytic cells containing either erythrocytes or pigment, or both, were found, and the impression was received that sufficiently extensive study would have revealed them in all. In seventy-seven out of the 130 cases the number of phagocytes containing either cells or pigment was not considered to be outside of what may be generally accepted as the normal limits. In twenty-one cases the phagocytosis was regarded as probably abnormal, and in thirty-two other cases the amount of phagocytosis undoubtedly exceeded the normal. In fourteen of the latter group pigment granules were present in the phagocytes to a relatively greater extent than were hemoglobin-containing erythrocytes. However, ingested red corpuscles were present in all specimens and usually in numbers greater than normal. The diagnoses in this group were — cirrhosis of the liver in three cases, hemachromatosis, adhesive pericarditis and perihepatitis, osteitis deformans and lobar pneumonia, lobar pneumonia and toxic jaundice, tuberculosis (two cases), carcinoma of esophagus, aplastic anemia and subdural hemorrhage, chronic myocarditis and chronic nephritis, actinomycosis, and Paget's disease. In twelve of the thirty-two cases there was a definitely excessive phagocytosis of erythrocytes, accompanied, in most instances, by a small or moderate number of hemosiderin granules. The diagnoses in this group were — bronchopneumonia or lobar pneumonia in six cases (with lung abscess and empyema in one case), typhoid fever, tuberculosis in two cases, mitral stenosis with thrombosis of left auricular appendage and multiple infarcts, and aplastic anemia and endometritis. One other case (24–112), which was studied incompletely in the clinic, as the patient died of a cerebral hemorrhage shortly after admission, is included here although the clinical record and the autopsy make it probable that he also had pernicious anemia. In the remaining six of the group of thirty-two cases both erythrocytes and pigment granules were present in the phagocytes to an abnormal extent. The diagnoses in these cases were — cirrhosis of the liver, chronic nephritis and healed perihepatitis, lobar pneumonia, bronchopneumonia and en-

cephalitis (?), gastric ulcer with perforation, and embolus of basilary artery. It is thus apparent that in a great variety of pathological conditions one may find a considerable number of erythrocytes and an abnormal amount of hemosiderin in the phagocytic cells of the bone marrow. It is impossible to classify these conditions, but the number of cases with liver lesions is rather striking. All five specimens of bone marrow from cases of cirrhosis of the liver showed red cells, pigment, or both, within the phagocytes. Beyond this one can only call attention to the incidence of increased phagocytosis in infections such as pneumonia, typhoid fever, and tuberculosis, in which the phagocytosis of cells is usually predominant. Similar findings have been described by Longcope[17] in typhoid fever and by Dickson[7] in pneumonia. While an excessive amount of phagocytosis was not observed in all of the cases of pneumonia or typhoid fever studied, it is of interest to call attention to the fact that certain cases of pneumonia and typhoid fever are associated clinically with slight degrees of jaundice, and Broun has found in some of these cases a "delayed" direct diazo reaction (Van den Bergh) which indicates that the jaundice is of hemolytic rather than of hepatic origin. It is quite probable that the hemolytic jaundice is the result of the phagocytosis. In cirrhosis of the liver Broun found the "prompt" direct diazo reaction, which is associated with jaundice of hepatic origin, but if the two types of jaundice, hepatic and hemolytic, were simultaneously present, the former would always mask the latter so that it would not be recognizable by this test.

An abnormal amount of phagocytosis is frequently observed in specimens of bone marrow which are hyperplastic, in the sense that there is an increase of cells at the expense of fat, but there is no definite relation between the degree of hyperplasia and the extent of the phagocytosis, and phagocytosis may be increased in specimens of bone marrow that are definitely not hyperplastic.

Pernicious Anemia

Eleven cases of pernicious anemia which have received careful clinical study form the basis of this report. Eight of them were patients at the Boston City Hospital and three at the Peter Bent Brigham Hospital. For the clinical records of the latter we are indebted to Dr. H. A. Christian, and for the pathological records and material to Dr. S. B. Wolbach. Ten of these cases may be said

to have died in an acute stage of the disease, although one of them (Case 2) also had a terminal pneumonia. The red corpuscle counts taken shortly before death varied between 500,000 and 1,000,000 per c.mm., and the hemoglobin variations were between 10 and 24 per cent in nine of the patients. In one case (Case 7) the hemoglobin was 55 per cent and the erythrocyte count was 1,200,000

TABLE 1

Cases of Pernicious Anemia

Case No.	Age	Per Cent Hemoglobin	Red Cells Million	Transfusions		Phagocytosis in Bone Marrow		Remarks
				Total Number	Last before Death	Hemosiderin	Erythrocytes	
1	64	19	1.0	2	14 days	+	+++++	
2	64	10	0.5	2	7 months	+	++++	
3	45	17	0.8	9	4 days	+	++++	Lobar pneumonia (terminal)
4	39	20	1.0	4	17 days	+	+++	
5	58	16	0.5	2	1 month	+	++++	
6	63	24	0.8	4	1 day	+	+++±	
7	55	55	1.2	0	....	+	+++±	Last hemoglobin determination was 13 days before death
8	65	10	0.8	1	5 weeks	+	+++±	
9	52	16	1.0	0	....	+	+++	
10	67	14	0.6	4	3 months	+±	+++±	
11	37	50	2.0	0	....	±	+	Lobar pneumonia

thirteen days before death. On the day before death the erythrocyte count was 1,080,000 per c.mm. The bilirubin in the blood plasma was between 1.3 and 4.0 mgm. per 100 c.c. of blood in four of the cases just before death, and was 0.8 and 2.0 mgm. in Cases 1 and 6 just after death. The eleventh case is considered separately from those dying in an acute stage of the disease because death was due to lobar pneumonia contracted during a remission of the pernicious anemia, at a time when the red cell count was 2,000,000 per c.mm. and the hemoglobin was 50 per cent. As will be seen, the histological findings in the bone marrow in this case were quite different from those in the cases dying in an acute stage of pernicious anemia.

Two important features characterize the phagocytic process in

the bone marrow in the first ten cases. These are the extraordinarily great amount of phagocytosis of erythrocytes and the relatively slight amount of hemosiderin present in the cells. The iron-staining pigment is not at all prominent and often scarcely exceeds the quantity that may be present in normal bone marrow. A part of it is deposited in very fine granules in the endothelium lining the vascular sinuses and a few small masses may be extracellular. The rest is in phagocytic cells, and is usually interspersed between the ingested red corpuscles. The extent to which erythrocytes have been phagocyted, on the other hand, is, as far as can be roughly estimated in eight cases, greater than in any of the control cases, and in two cases about equal in degree to the half dozen control cases which showed the largest amount of phagocytosis of cells. With one exception, however, (24–112, probable pernicious anemia) all of these control cases showed much more pigment than did the cases of pernicious anemia.

The phagocytosis of erythrocytes usually takes place in free, round or oval cells (endothelial leucocytes; clasmatocytes) with large vesicular nuclei which become compressed, often curved, and displaced toward the periphery of the cell when many red corpuscles have been ingested. If a single erythrocyte has been ingested it often lies within the concavity of the nucleus. Phagocytosis may also occur in cells with slightly eosinophilic cytoplasm and delicate cytoplasmic processes. The number of phagocytic cells varies in different parts of the same section but there are often as many as three to six in a single high-power field. The number of erythrocytes ingested by a single phagocyte is also very variable and runs from one to three up to twenty or more. When one takes into consideration the fact that in pernicious anemia the cellular, hyperplastic bone marrow is a wide-spread, extensive tissue which fills the long bones as well as the short bones and which even brings about an enlargement of the marrow cavities within the long bones, it is apparent that the number of erythrocytes which are being phagocyted is enormous. The extent of the phagocytosis is indicated by Figures 1 to 4, each of which is from a drawing of the cells in a single oil-immersion field.

The ingested erythrocytes are for the most part strikingly normal in their appearance, both as regards size and shape. Small, abnormal, irregular forms and poikilocytes are often seen but they are,

on the whole, less common than normal red corpuscles. The hemoglobin usually stains exactly as does the hemoglobin in the red cells in the vascular spaces. Erythrocytes in which the pigment has been altered so that it has the appearance of hemosiderin or gives an iron stain are distinctly rare. Nucleated erythrocytes are very frequently found within phagocytes and occasionally leucocytes have been ingested.

The phagocytic cells described above are seen best in well-stained thin sections of bone marrow. They are, however, often somewhat difficult to distinguish in the densely cellular tissue and may be more easily recognized along the edge of the sections where the cells have been mechanically separated. In spite of being present in large numbers, they may well be overlooked in a routine study of the bone marrow unless attention is directed to this particular feature. The phagocytic cells are also readily found in fresh smears or in smears made from fresh emulsions of marrow in salt solution. They are quickly destroyed in smears dried in the air before fixation, but excellent preparations may be obtained by fixing the smears while still wet in Zenker's solution and then staining. Dickson [7] recommends this method of examination. Very satisfactory preparations may also be made by fixing in Zenker's solution the sediment of an emulsion of bone marrow in salt solution. These "wet fixed" smears may be stained with eosin and methylene blue or with one of the Romanowsky stains. The phagocytic cells are also easily seen in fresh, unstained emulsions of bone marrow in normal salt solution, and Dr. C. A. Doan has recently made a beautiful preparation of living bone marrow cells stained with neutral red and Janus green in which the phagocytic cells were particularly prominent.

Case 11, who, as mentioned above, died of pneumonia during a remission in the pernicious anemia, and in whom the relative inactivity of the process causing the anemia is indicated by a red corpuscle count of 2,000,000 per c.mm., and a blood bilirubin content of only 0.8 milligrams per 100 c.c., showed a histological picture in the bone marrow which was quite different from that described in the ten cases just referred to. There was very little phagocytosis either of erythrocytes or of pigment. The degree of phagocytosis in a single section could hardly be considered beyond the normal limits, although, of course, the process was going on throughout a tissue that is somewhat more extensive than the normal bone mar-

row. In this connection another point in the histology may be mentioned. Eight of the ten cases dying in an acute stage of pernicious anemia were almost without fat in the bone marrow, while two others (Cases 4 and 10) had only a slight amount of fat in the marrow. Case 11, on the other hand, had a very considerable amount of fat in the femoral bone marrow. This is unquestionably another indication that the pernicious anemia was undergoing a remission, for Zadek [18] has shown by examination of the marrow during life that during remissions in the disease the bone marrow tends to become more yellowish and less red in color.

Discussion

The observations on the bone marrow which have just been described indicate that phagocytosis of erythrocytes occurs to a very limited extent in normal marrow; that it may be considerably increased in a variety of pathological conditions; that in the active stages of pernicious anemia it occurs to a degree which, with rare exceptions, is not met with in other diseases, and that during a remission in the course of pernicious anemia it may cease to be a prominent factor in the bone marrow.

The first point for discussion with regard to the phagocytosis of erythrocytes in pernicious anemia is as to the character of the ingested cells. Are they foreign cells which have recently been introduced by transfusion? That they are not transfused cells is evident for a number of reasons: extensive phagocytosis of erythrocytes was frequently described long before transfusion became a common procedure; many of the ingested erythrocytes are young cells that still retain their nuclei; Case 2 of this series, with an enormous amount of phagocytosis, had not been transfused for nearly seven months; and Cases 7 and 9 had never been transfused. It is also of interest in this connection that in one of Dr. Channing Frothingham's patients who had received thirty-two transfusions, the bone marrow showed no phagocytosis of erythrocytes, but contained a very large amount of intracellular hemosiderin. The second question with regard to the character of the ingested erythrocytes is less easy to answer. Are they abnormal or inadequate cells? Is the phagocytosis merely a mechanism for ridding the circulation of worthless cells? The fact that the ingested cells look like entirely normal erythrocytes is,

of course, no evidence as to their functional efficiency. The occurrence, however, of so many nucleated red corpuscles among the phagocyted cells indicates that if these cells are indeed inadequate, they must become so very early. This is opposed to the observation of Wearn, Ames, and Warren [19] that the cells of a patient with pernicious anemia, transfused into another subject, continue to remain in the circulation as long as transfused normal cells. Such evidence certainly does not suggest that the erythrocytes in pernicious anemia are, as a whole, in any degree inadequate, but whether or not some of the red cells are rendered more liable than normal to phagocytosis in the patient with pernicious anemia is not known. The phagocytosis of erythrocytes in pernicious anemia must depend either on an alteration in the red cells which makes them more readily phagocyted or on a stimulus to the phagocytic cells which increases their avidity for erythrocytes. No convincing proof of either process can, however, be brought forward at the present time.

From the point of view of pernicious anemia the problem of immediate interest is whether this phagocytic process is an incidental matter, as it has generally been regarded, or whether it is a significant phase of the pathology of the disease. If the phagocytic process does have an essential relation to the pathology of the disease, this relation is, of course, limited to the hematological manifestations of the disease, namely, the anemia and the hemolytic jaundice. No other explanation of the cause of the anemia has been presented which is in any degree convincing and there are, on the other hand, certain facts which indicate that the phagocytosis may play a part in its production. The most striking of these is that in certain cases of pernicious anemia there is an easily demonstrated phagocytosis of erythrocytes in the bone marrow which is rarely equaled in degree in other pathological conditions. There can be no doubt that the phagocyted cells are ultimately destroyed and the amount of phagocytosis throughout the greatly increased bone marrow must account for the destruction of a very large number of erythrocytes. The histological picture of the bone marrow, with extensive phagocytosis of hemoglobin-containing erythrocytes, and with very little retention of hemosiderin in the phagocytes suggests an active hemolytic process. The hemoglobin or its decomposition products immediately after their formation probably pass quickly from the phagocyte into the blood stream. This would account for the finding

of hemoglobin and hematin, as well as bilirubin, in the plasma in acute stages of pernicious anemia, as reported by Broun, Ames, Warren, and Peabody.[20] Unfortunately the group of cases thus far studied satisfactorily, both clinically and pathologically, is extremely small but it is significant that the cases dying during an acute phase of the disease showed the greatest amount of phagocytosis of erythrocytes, while the case dying of pneumonia during a remission of the anemia showed by far the least amount of phagocytosis.

In this paper attention has been directed entirely to the phagocytosis of erythrocytes in the bone marrow. The bone marrow has been considered to be of particular interest because it is so large an organ in pernicious anemia, and also because phagocytosis is, at least as demonstrated histologically, a less considerable feature in the liver and spleen in this disease. The failure to cure pernicious anemia by splenectomy also indicates that the spleen plays, at most, a subordinate rôle in the production of the anemia. The bone marrow is not the only tissue, however, in which extensive destruction of red corpuscles takes place in pernicious anemia. In 1902 Warthin[21] published a paper on "The Pathology of Pernicious Anemia with Special Reference to Changes Occurring in the Hemolymph Nodes," and described extensive phagocytosis of erythrocytes in eight cases. One of his conclusions may well be quoted verbatim as it summarizes his point of view as to the hemolytic process in pernicious anemia. "The poison of pernicious anemia stimulates the phagocytes of the spleen, lymph and hemolymph glands, and bone marrow to increased hemolysis (cellular hemolysis). Either the phagocytes are directly stimulated to increased destruction of red cells, or the latter are so changed by the poison that they themselves stimulate the phagocytes. The hemolysis of pernicious anemia differs only in degree, not in kind, from normal hemolysis or the pathological increase occurring in sepsis, typhoid, etc."

Our own observations on the lymph nodes in pernicious anemia have, as yet, been limited in number but they are in entire agreement with those reported by Warthin. The studies on the bone marrow, described in this paper, taken in conjunction with the observations of Warthin, lend considerable support to his explanation of blood destruction in pernicious anemia by cellular hemolysis. Whether or not this method of blood destruction is the only factor in the production of the anemia, of course, remains an unsettled

question. It is quite possible that the hemolytic jaundice of pernicious anemia is the result of phagocytic action but that other factors take part in the causation of the anemia.

Conclusions

1. Phagocytosis of erythrocytes occurs in the normal bone marrow and to a much greater extent in a variety of pathological conditions. Among the latter the most notable are certain cases of cirrhosis of the liver, and of infectious diseases such as pneumonia, typhoid fever, and tuberculosis.

2. Phagocytosis of erythrocytes occurs in the bone marrow of patients dying in an acute stage of pernicious anemia in a degree rarely met with in other conditions. In a single case of pernicious anemia dying in a remission of the disease, phagocytosis of red blood corpuscles was not a striking phenomenon.

3. It is suggested that phagocytosis of erythrocytes may be a factor in the production of the hemolytic jaundice and of the anemia in pernicious anemia.

It would have been impossible for us to carry out this study without the very constant assistance of Dr. F. B. Mallory, Director of the Pathological Laboratory, and his group of associaies. For their many kindnesses we are glad to express our deep appreciation.

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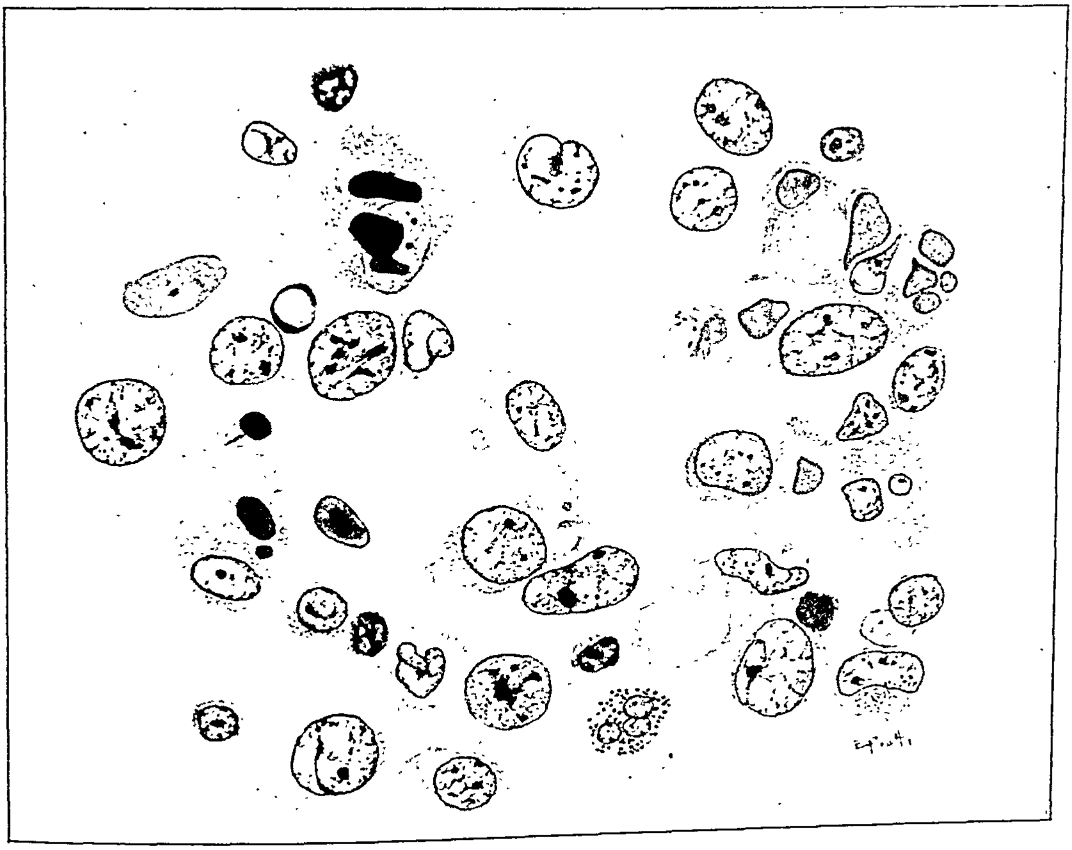
DESCRIPTION OF PLATES XXIX AND XXX

The illustrations are photographs, from drawings by *Miss E. Piotti, of cells* found in a single oil-immersion field.

Figures 1 and 2 are from smears of femoral bone marrow, and Figures 3 and 4 are from sections of femoral bone marrow.



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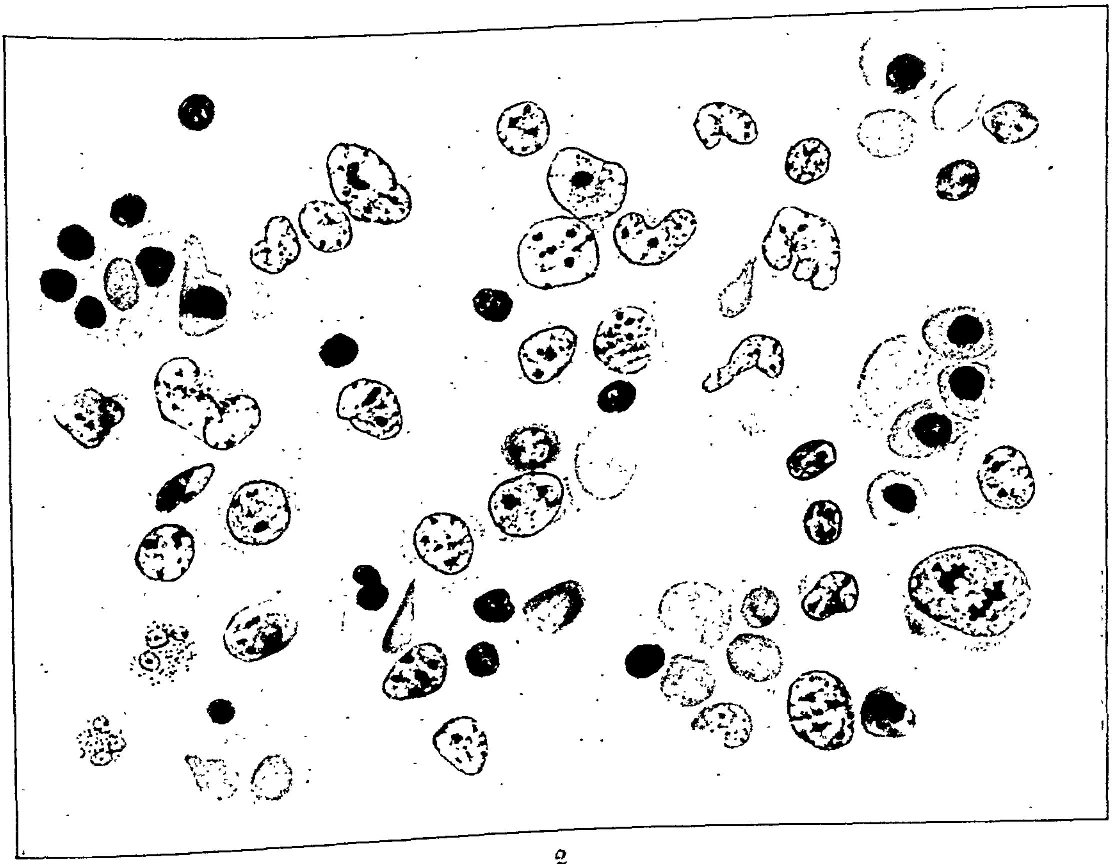
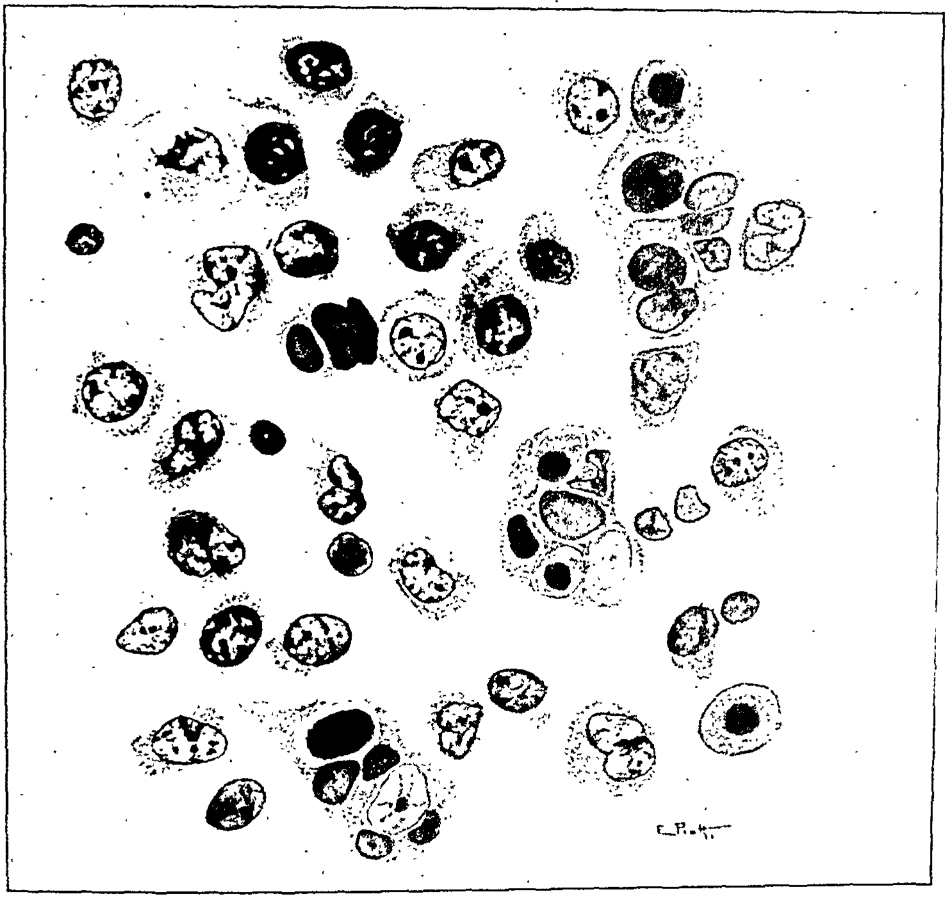


2

Phagocytosis of erythrocytes

Peabody and Broun



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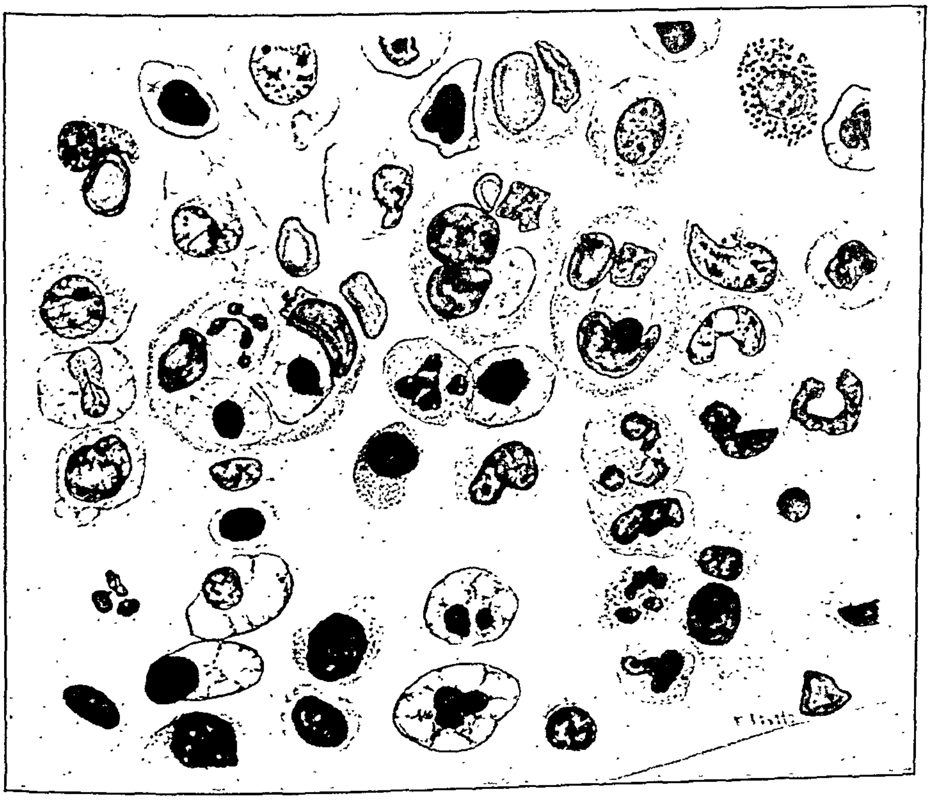

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Peabody and Broun Phagocytosis of erythrocytes

CONCERNING THE MICROSCOPIC STRUCTURE OF THE HYPOPHYSIS CEREBRI IN ACROMEGALY*

(Based on a study of tissues removed at operation from 35 patients)

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If acromegaly is a disease entity, whether the lesion in the hypophysis be primary or secondary, the adenomatous change which takes place in this gland should show some constant histological characteristics capable of recognition. For the purpose of identifying these characteristics, if possible, and thereby explaining or interpreting the contradictions in the literature, we felt it would be fruitful carefully to re-examine the abundant material collected over a period of years in this clinic.

It is, of course, a fundamental principle that the microscopic investigation of the pathologic alterations of an organ demands a knowledge of its normal microscopic structure, which implies the use of certain special technical procedures by which the normal structure is best displayed. It is not sufficient to employ, as is still often done, simply stains to show nuclei and certain pathological substances such as fat. Many excellent methods for the study of the normal microscopic structure of the hypophysis have been developed, and although perhaps none of them is perfect in every respect, their neglect is doubtless the cause of much of the uncertainty and contradiction regarding the acromegalic gland.

Normal Microscopic Details

It is a generally accepted view that the part of the hypophysis affected in acromegaly is the anterior lobe (*pars distalis*) and, in accordance with the principle just mentioned, a knowledge of its normal structural peculiarities is necessary. It may be said, at the risk of repeating common knowledge, that the pars distalis is composed of polygonal cells having, for the most part, sharply defined

* Read before the Boston Society of Neurology and Psychiatry, February 21, 1924. Received for publication December 26, 1924.

cellular boundaries. They are arranged in thick columns, between which wind delicate vascular sinuses.

The cells of the pars distalis are distinguished, by the type of granules which they contain, into acidophilic, basophilic and chromophobe cells. This terminology was based on the belief that one type of granules has an affinity for acid dyes and the other for basic dyes. It is now known that this belief was erroneous. It is preferable to speak of the granules as α and β granules, the α being easily understood to mean the acidophilic and the β to refer to the so-called basophilic (cyanophilic) granules. The cells which contain these granules may then be spoken of as α or β cells, for cells containing both types are not known to exist under normal conditions. The α and β cells are spoken of collectively as chromophile cells.

In addition, there are cells in the pars distalis which contain no granules. Some of them are chromophile cells which have lost their granules, but most of them have apparently failed to develop granules and are known variously as chromophobe, reserve, or chief cells. They often lie in the center of the cellular columns, although they may make up entire columns, especially in the periphery of the pars distalis or near the infundibulum. Often these reserve cells do not have distinct boundaries and their nuclei lying close together are known as nuclear clusters (Kernhaufen).

The α granules are large and distinct. They are stained intensely by eosin, acid fuchsin, neutral ethyl violet, etc. The β granules are finer and less distinct. They are also more difficult to stain, but alum hematoxylin, kresofuchsin, acid violet and aniline blue are capable of displaying them. There are also granules of a general nature, such as mitochondria and lipoid droplets, which may be demonstrated by special means.

The relative numbers of α and β cells in different hypophyses is most variable, even in different parts of the same pars distalis. The pattern which one finds may change completely in the space of a dozen serial sections. The relationship of the different cellular types to the blood vessels shows no constancy whatever.

Technical Procedures

All of our specimens were fixed immediately after removal from the body. The best fixative for the purpose is undoubtedly Regaud's fluid. Formalin-fixed material may be used if it is mordanted for

10 days in a 3 per cent aqueous solution of potassium bichromate before it is embedded. Alcohol, acids and sublimate mixtures are unsuitable. Acids (except osmic acid) and alcohol agglomerate the α granules, which are lipoidal, and prevent the demonstration of the finest ones, while sublimate produces artificial granulation of the cytoplasm which is apt to simulate β granules.

The α granules may be stained by many methods, among which may be mentioned: Benda's toluidin blue-sodium sulphalizerinate; methylviolet (M. B. Schmidt, Bizzozero-Launois); Heidenhain's or Regaud's iron hematoxylin; phosphotungstic acid-hematoxylin of Mallory; Kraus' hematoxylin; acid fuchsin of Altmann or Cagnetto; neutral gentian (Lewis); and neutral ethylviolet-orange G (Bailey). Eosin is totally unreliable for the identification of α granules. The best stain is neutral ethylviolet-orange G. It gives a sharply specific stain, a sharp contrast, and will not stain mitochondria. On material fixed in Regaud's fluid, Altmann's acid fuchsin and iron hematoxylin will always stain the mitochondria along with the α granules.

For the demonstration of the β granules on material fixed in Regaud's fluid only acid violet has been found to give a sharp stain (Bailey). On material fixed in formalin, kresofuchsin may be used. For specimens fixed in Zenker's fluid we have used alum hematoxylin.

For a sharp differential stain of the two types of granules in the same preparation we prefer the acid fuchsin-acid violet method of Bailey.[1] The neutral safranin-acid violet method of Lewis and Maurer[28] may be used on Regaud-fixed material provided the sections are passed for 3 minutes into 1 per cent aqueous solution of potassium permanganate and bleached in oxalic acid before being stained. We prefer the procedure of Lewis and Maurer to the earlier one of Bailey, in which the same combination of dyes is used.

The method described by Bailey for the differentiation of α granules and mitochondria in the same preparation has been found unreliable and abandoned. No other has been found to replace it. We have been obliged to depend upon a comparison of acid fuchsin and ethylviolet-stained preparations.

It has been necessary to use some material fixed in Zenker's fluid. For the identification of β granules in these tissues we have used alum hematoxylin. Since it errs on the side of too many

rather than too few granules, we feel certain that they are absent from our material. Many of the finer α granules are completely lost from Zenker-fixed material. We proved this conclusively and repeatedly where we had specimens fixed both in Zenker's fluid and in 10 per cent formalin from the same case. In the formalin-fixed material we could easily demonstrate α granules, while in the Zenker-fixed material they could be shown only incompletely with any of the methods at our disposal. We found, however, that the granules came out more sharply if the sections were mordanted in 3 per cent potassium bichromate before being stained.

Lack of a proper knowledge of staining methods explains many strange errors in the literature of acromegaly. Launois [29] gives in great detail a method of using methyl violet after Bizzozero, which he claims is a specific stain for β granules. Kon [25] falls into the same error with the method of M. B. Schmidt. Both methods stain α granules sharply and specifically as can be easily proved by trying them on an acromegalic adenoma known to contain only α granules. Huchard and Launois presumably used this method on their giant (see below, p. 200) thus unconsciously entering this case in the ranks of those having a pure eosinophilic adenoma, while in the literature it is often quoted, erroneously, of course, as the only acromegalic in whom a basophilic adenoma was found. St. Rémy [30] and Bartlett [12] have fallen into the error of confusing α granules and mitochondria, because they used Altmann's acid fuchsin. The latter described α granules in the cells of a tumor of the hypophysis from a case not clinically acromegaly. The granules he saw were doubtless mitochondria.

Although some of our material was unfortunately fixed in Zenker's fluid or in Bensley's fluid,* both of which are unsuited to the hypophysis, most of the specimens were fixed in 10 per cent formalin, and this material, after being mordanted in 3 per cent potassium bichromate, is serviceable. All the specimens since 1920 were fixed in Regaud's fluid.

This report is based upon a study of the tissues removed at operation from thirty-five patients. This number includes all but

* It consists of equal parts of (1) saturate solution of mercury bichloride in 95 per cent alcohol and (2) 2.5 per cent aqueous solution of potassium bichromate. It is one of many formulae proposed by Bensley but is known in the pathological laboratory as Bensley's fluid and we shall continue to speak of it under this name.

three of the patients with acromegaly operated upon by Dr. Cushing at the Peter Bent Brigham Hospital. The material removed from two of the other three patients has been lost and that from the third was so imperfectly fixed that it was impossible to use it. In every case the diagnosis of acromegaly, or of dyspituitrism with acromegalic manifestations, was clearly established from the clinical examination alone, before the information obtained from microscopic examination.

As controls we have used the adenomas from fifty-four patients showing dyspituitrism without acromegalic manifestations. This includes all the cases operated upon in this clinic since 1920 from which the tissue removed was fixed in Regaud's fluid. (These statistics have been *completed up to Nov. 1, 1924.*)

Characteristics of Acromegalic Hypophysis

It might be well to state briefly at this point the results of our investigations, namely, the characteristics which distinguish the microscopic structure of the pars distalis in acromegaly from that of the normal hypophysis and from that of the adenomatous enlargement unaccompanied by acromegaly. These distinguishing characteristics may readily be appreciated from the series of figures in Plate XXXI.

In Fig. 1 the normal pars distalis may be seen to consist of columns of polygonal cells separated by delicate vascular sinuses. The cells consist of α and β chromophile cells, and chromophobe or reserve cells.

The pars distalis shown in Fig. 2 from a case of adenomatous enlargement *with* acromegaly is composed of a mass of rounded or polygonal cells, almost without blood supply. The cells are α cells containing α granules which are finer than normal and have a tendency to collect around the periphery of the cytoplasm. No β cells are present. Some of the cells may be without granules (Entgranuliertenzellen of Kraus). The size of the cells varies greatly, and multinucleated cells are numerous; some cells may have more than half a dozen nuclei. Amitotic nuclear divisions are commonly seen. Many cells are flattened onto the surface of adjacent cells in the form of a crescent (cf. Plate XXXII, Fig. 1).

The pars distalis in a case of adenomatous enlargement *without* acromegaly is shown by Fig. 3 to be composed of very thick columns

of cells separated by thin septa of connective tissue, carrying delicate capillaries. The cells are elongated, epithelial cells and are all chromophobe. Each cell has a simple elongated nucleus. The cells contain no other granules than mitochondria. The cell boundaries are often very indistinct (cf. Plate XXXII, Fig. 2).

Naturally all transitions are encountered between these three types of structure.

Case Reports

It is impossible and unnecessary to submit detailed reports of all the thirty-five patients. Four characteristic cases have been selected. Three of the patients were typical acromegalics, one having some evidence of gigantism; the fourth had a dyspituitary syndrome with marked acromegalic features. The tissues from these four cases were variously fixed — from the first in Zenker's fluid, from the second in formalin, from the third in Bensley's fluid and from the fourth in Regaud's fluid.

Realizing that many of the cases reported in the literature as acromegaly are doubtful examples of this disease, sufficient evidence will be presented, it is hoped, to establish the clinical diagnosis of our cases beyond question. Since the diagnosis of this disease depends so largely on bony changes and the external appearance of the patient, Roentgenograms and photographs are given, although the best photograph translates but imperfectly the impression one gets by direct observation, and in reproduction also many details are lost.

Case I

This patient entered the clinic at a time when it was the custom to fix in Zenker's fluid the tissues removed at operation.

Surg. No. 496. Acromegaly. Operation. Recovery.

October 16, 1913, Mr. C. M. W., an unmarried English solicitor, aged 24, was admitted, complaining of developmental changes and drowsiness.

The patient's father was 6 feet tall, but showed no disproportionate enlargement of the extremities. The patient had pertussis and scarlatina at 15, and pleurisy with effusion at 16. After recovering from this illness he began suddenly and rapidly to grow taller, so that in the 5 years between 16 and 21, his stature increased 8 inches. During the past 3 years he had grown little in height, but his hands and feet had enlarged markedly. Gradually over a period of 7 years, he had developed a host of symptoms such as failing memory, epiphora, polyphagia, constipation, hyperhydrosis, drowsiness, dull aching head pains, intermittent pains in the arms and legs, decreasing libido sexualis, failing vision, diplopia, definite subjective bitemporal hemianopsia, hallucinations of smell,

and marked protrusion of the lower jaw. The order of development of many of these symptoms is uncertain, but he had noticed the prognathism almost from the beginning.

On admission his height was 200 cm.; weight 108.6 kg. He was a strikingly tall, gigantic man, though listless in appearance and failing to make an impression of prowess corresponding to his stature. His skin was moist with perspiration and quite elastic. The hair on his head was fine in texture and luxuriant, but in other parts the growth of hair was scanty. All the bones of his skeleton were heavy and thick, especially the enlarged epiphyses. His arms and legs were particularly massive, with good muscular development. But the hands

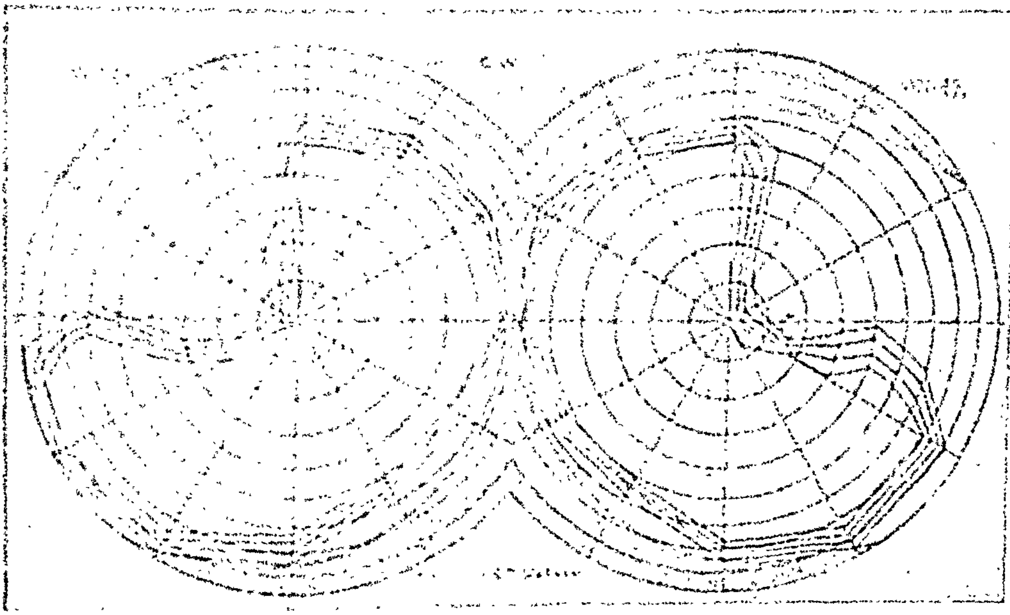

FIG. 1. Visual fields of patient I.

and feet were enormous. They were thick, soft, spadelike. The most marked enlargement of the head was shown in the frontal and facial bones. The eyes were wide apart and deeply set; the nares wide; the lips thick and heavy. The tongue was coarse and large; the teeth were widely spaced; the lower jaw was markedly prognathic (Plate XXXIII). The thyroid gland and genitalia were normal. The optic discs were pale and somewhat hazy.

His blood pressure was 120/74. Routine blood and urine examinations were normal. No metabolism determination was made. The visual fields showed characteristic defects in the upper two-thirds of both temporal regions (text Fig. 1). X-ray examination disclosed a moderate thickening of the frontal bone, an enlarged sella (Plate XXXV, Fig. 1), an overdeveloped occipital protuberance, an enormous frontal sinus, and marked hypertrophy of the mandible. The distal phalanges of both hands (Plate XXXIX, Fig. 1) and feet showed marked exostoses, and some tendency to bony overgrowth was apparent around the metatarso-phalangeal joints.

On October 20, 1913, Dr. Cushing exposed the floor of the sella turcica by the transphenoidal approach. The sellar floor was of dense bone which was chipped away with some difficulty and a smaller decompression than usual secured. A

crucial incision was made in the exposed dural capsule, and a few fragments of the adenoma were removed.

He recovered promptly from the operation and was discharged November 5, 1913, with some early improvement in his visual fields. He subsequently saw service with the British Army in France and on May 6, 1922, last wrote that his malady appeared to be stationary.

Histological examination of tissue removed at operation

The tissue was fixed in Zenker's fluid and stained with hematoxylin and eosin. As no fresh sections were available, one of the original sections was restained with neutral ethylviolet-orange G (Plate XL, Fig. 1).

The tissue consists of polygonal tumor-cells with very few blood vessels and scanty connective tissue. The cells vary greatly in size. They have distinct cell-boundaries and large vesicular nuclei, several of which are often contained in one cell. Crescent formation may be seen.

Many cells contain α granules tending to congregate in the periphery of the cytoplasm. Because of the imperfect method of preparation it is impossible to determine whether all the cells possess this granulation, but it is obvious that most of them have some α granules. β granules were not found.

The tissues from twenty-two other patients have been fixed in Zenker's fluid or in formol-Zenker. This fixation is not the best, in our opinion, but tissues so fixed may be used as will be shown. In the following case formalin was used as a fixative.

Case II

Surg. No. 826. Dyspituitrism with marked acromegalic manifestations. Operation. Death from meningitis. Necropsy.

January 23, 1914. Mr. J. H. D., a school teacher of 46, was admitted complaining of skeletal overgrowth, adiposity, drowsiness and failing vision. Referred by Dr. J. W. Courtney of Boston, Mass.

The patient's father and 8 siblings were of unusual stature. He had been married for 21 years and had 9 children, the youngest being 4 years of age. He had always been healthy. He reached a height of 5 feet 10 inches at the age of 22, and weighed 170 lbs. He maintained this height and weight until his present illness.

The onset of his present illness was gradual. He noticed a gradual gain in weight and increasing size of his hands, feet and features 8 years before. About a year later he began to feel drowsy and became markedly constipated. About 4 years before admission his memory became poor and his speech indistinct. At the age of 44 he noticed rather suddenly that vision in his right eye was imperfect. During the past year his left eye became similarly affected. Beginning about two years ago his libido sexualis diminished until at admission it was entirely absent.

Examination showed a large man whose height was 179 cm. and weight 110 kg. He was drowsy; he had a defective memory; and his speech was indistinct because of his large tongue. The skin was coarse and elastic over the extremities; the palms and soles were quite moist. Numerous fibromata mollusca were present over the neck and chest. The hair was normally abundant and coarse,

but pubic hair showed a feminine distribution. The extremities were characteristic of a markedly advanced acromegalic state (Plate XXXIV). There was no prognathism. The optic discs were pale, with sharp outlines. The thyroid gland was not palpably enlarged. Blood pressure was 132 mm. Hg.

The X-ray disclosed a ballooned-out sella turcica with erosion of its bony walls (Plate XXXV, Fig. 2). The terminal phalanges showed marked tufting (Plate XXXIX, Fig. 2). A left homonymous defect, more advanced in the right eye, was present in the visual fields (text Fig. 2). His temperature while under observation tended to be subnormal and there was a slight polyuria but no glycosuria. The blood Wassermann reaction, routine blood and urine examinations were negative. He left the hospital after 5 days' observation to return again the 21st of February, 1916 (Surg. No. 4310).

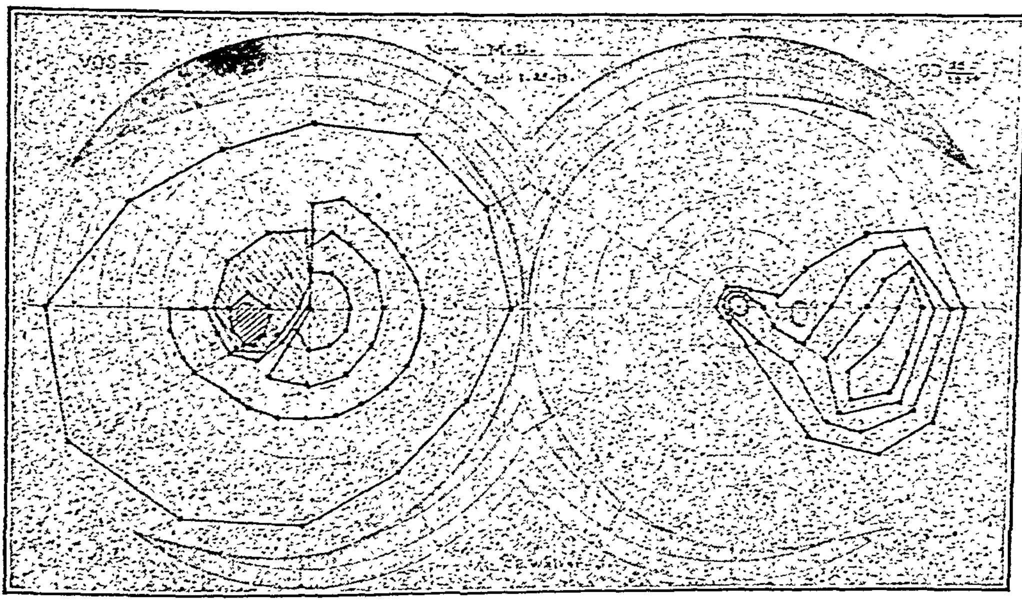

FIG. 2. Visual fields of patient II.

During the interval he had gained in weight 5.5 kg. His vision had decreased perceptibly, and examination of the visual fields showed an increase in the field defects. His basal metabolism was now examined for the first time and was found to be + 22.

On March 3, 1916, Dr. Cushing did a transphenoidal operation and found that the tumor had already completely eroded the floor of the sella and extended into the sphenoidal sinuses. The thin and distended capsule of the tumor was incised and the soft growth extruded itself in large masses. More material was removed with the pituitary spoon. The tumor was somewhat more vascular than usual.

The patient recovered well from the immediate effects of the operation, but five days later his temperature suddenly rose to 104.2° and in a few hours he became rapidly comatose and died.

A necropsy limited to the head was performed 7¾ hours post-mortem. A large tumor mass occupied the region of the sella turcica. Projecting from its lower surface was an additional mass of tumor which had herniated through the

operative incision of the capsule. The carotid arteries were widely separated by the tumor, and the optic nerves were angulated by a band of dura and greatly flattened by the upward pressure from the growth, which extended upward for about 3.5 cm. above the sellar floor. The tumor mass was rather firm and quite black (after formalin fixation), suggesting hemorrhagic infiltration. There was no gross evidence of meningitis.

Histological examination of tissues removed at operation

Tissue fixed in 10 per cent formalin and mordanted in 3 per cent potassium bichromate for 8 days, stained with neutral ethylviolet-orange G, acid violet-eosin, and hematoxylin and eosin. The tumor consists of a structureless mass of polygonal cells and a very few thin-walled blood vessels. The cells and nuclei vary greatly in size. The cytoplasm is rather scanty and only a few cells with more than one nucleus are seen. Many of the cells contain clear-cut α granules, usually disposed in the periphery of the cell (Plate XL, Fig. 2). No β granules are present.

Histological examination of tumor removed post-mortem

Three blocks were cut from the formalin-fixed tumor — one from the sellar contents, and two from the upper portion. They were mordanted for 8 days in 3 per cent potassium bichromate solution, cut, and stained with neutral ethyl-violet-orange G, acid fuchsin-methyl green, acid fuchsin-acid violet, and hematoxylin and eosin.

The structure is largely similar to that found in the tissues removed at operation, except that a larger proportion of the cells contain α granules.

An attempt to find a more suitable fixative was made by introducing a formula proposed by Bensley, and the tissues from the following patient were fixed in this way.

Case III.

Surg. No. 1664. Acromegaly. Operation. Recovery.

August 19, 1914, M. J. C., an unmarried school teacher of 25, was admitted complaining of headaches, drowsiness, overgrowth and eye trouble. Referred by Dr. J. B. Mason of London, Ky.

He came of a family of tall people. His father was 6 feet tall. His paternal great uncle was 7 feet 1½ inches tall and was exhibited in a traveling circus under the title of the "Kentucky giant."

The patient had typhoid fever at the age of 7 years. His secondary sex characters appeared at the age of 14. When 16 years of age he first noticed a prominence of the forehead, and an increase in the size of his hands and feet and lower jaw. At 19 he suffered from severe night sweats, which still continue. At 20 he began to have severe, dull, occipital headaches; during one period it seemed as though he were losing his eyesight, but this subsequently improved. Three to four years before admission he noticed a markedly increased hunger with especial appetite for sweets, increased thirst, and increased urinary output. For the past two years he had been somewhat somnolent, even in the daytime, and had become fatigued very easily. His weight at 17 was 180 pounds; at 19, 190; at 20, 212; and at admission 197 pounds.

Examination showed a tall, heavy man of strikingly acromegalic type (Plate XXXVI). His height was 175.5 cm.; weight 90 kg. The skin was somewhat sallow, moist, very elastic, and several pigmented moles were present over the body. The hair of the scalp and beard was coarse and heavy, but normal everywhere else. The thyroid gland and external genitalia showed no evident abnormalities. There was slight bilateral primary atrophy of the optic discs.

His blood pressure was 155/95. X-ray films of the skull showed marked prognathism and enormous frontal sinuses. The sella turcica was much enlarged, its floor depressed, and the dorsum sellae very thin (Plate XXXVIII, Fig. 1). Films of the hands showed marked terminal "tufting" of the phalanges

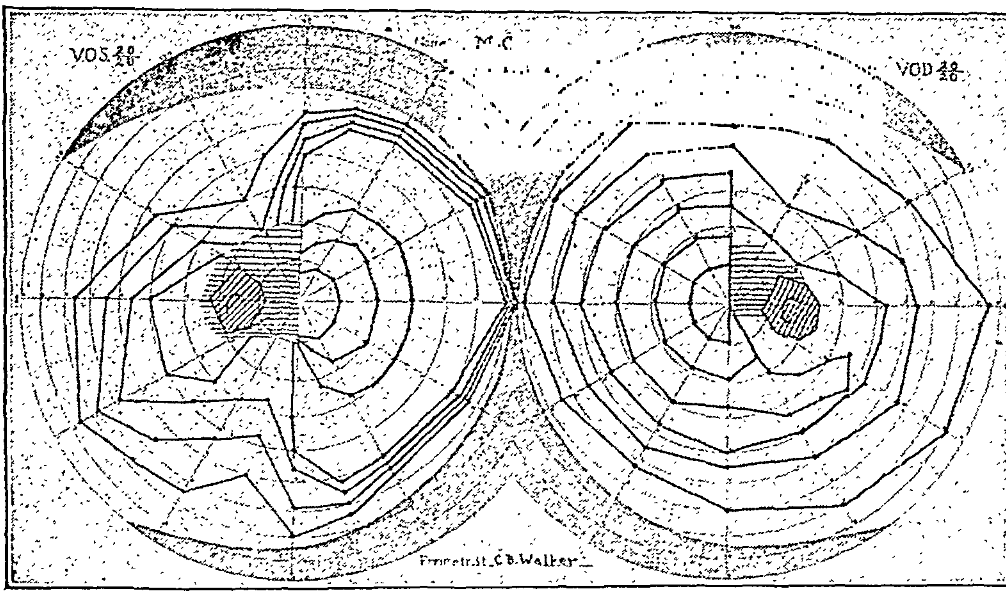

Fig. 3. Visual fields of patient III.

(Plate XXXIX, Fig. 3). There seemed to be no remaining evidence of the epiphyseal cartilages. Visual fields showed bitemporal defect with 20/20 vision (text Fig. 3). His temperature was normal and there was no polyuria. Marked hyperhidrosis was present.

Blood, urine and Wassermann tests were negative. His alimentary sugar tolerance was over 250 grams of glucose. No metabolism estimation was made.

On August 22, 1914, Dr. Cushing exposed the sella turcica by a transphenoidal approach. When the dural capsule over the tumor was incised, soft yellowish tumor-tissue extruded itself through the opening. A considerable amount of this tissue was spooned away.

He recovered rapidly and was discharged August 29, 1914. His headaches were relieved and slight improvement had occurred in central vision. He reported on April 21, 1922, that his condition was not markedly changed except that his lower jaw was perceptibly larger. Though his vision is retained, he has been obliged to give up his teaching work.

Histological examination of tissue removed at operation

Tissue fixed in Bensley's fluid and stained with neutral ethylviolet-orange G, acid violet-eosin, and hematoxylin and eosin.

The tumor consists of a structureless mass of cells with practically no supporting tissue except in the thin walls of the rare blood capillaries. The cells have very definite cell-boundaries and vary greatly in size and shape. The nuclei are vesicular and many cells contain from two to six of them. Crescent formation is occasionally seen.

The cytoplasm of these cells is packed with α granules (Plate XL, Fig. 3). No β granules are to be found.

Undoubtedly the best fixative for the hypophysis is Regaud's fluid, and since 1920 this fixative has been employed as a routine for all hypophysial adenomas. The following case was chosen because it is an example of early and slight acromegaly. It is even doubtful whether one could be sure of the diagnosis from the photographs given here. Yet there was no doubt of the diagnosis in the minds of any of us who saw the patient in this clinic, and microscopic examination of the tissues removed at operation showed a typical picture of what we have described here as acromegalic adenoma.

Case IV

Surg. No. 22532. Acromegaly. Operation. Recovery.

November 4, 1924, admission of Vivian H., a clerk, 22 years of age, complaining of headache, failing vision, and enlargement of the hands and feet. Referred by Dr. R. T. Woodyatt of Chicago, Ill.

There was no history of unusual stature or of any other developmental anomalies in the family. The patient had never suffered from any serious illness. Her menses began at the age of 13, and were always regular until November, 1923. Since that date they were irregular and scanty until April, 1924, when they ceased completely.

The present illness began about two years before admission to the hospital, with a feeling of discomfort over the left eye and occasional slight bitemporal headache. The headache had steadily increased in frequency and was now present practically every day. Accompanying the headache would occasionally appear oedema, swelling and pain around the left eye. One year before her menses became irregular, and she noted that she had to wear larger gloves. She had noticed no change in her features. Profuse perspiration had annoyed her for the last six or eight months. Her vision began to fail in June. In the last 2 years she had gained about 12 pounds in weight.

The patient had been given a series of 7 Roentgen-ray treatments since July, 1924, but her vision continued to fail, so she was sent to this clinic.

She was a strong, active young woman; not at all obese. There was some coarseness of the nose and lips which the patient herself had not noticed. It is scarcely noticeable in the photograph (Plate XXXVII), but comparison with photographs taken four years earlier leaves no doubt of its presence. There was no prognathism. The hands were typically acromegalic, having thick, square fingers and moist palms. There was little deformity of the feet. Roentgenographs show an enlarged sella turcica (Plate XXXVIII, Fig. 2). There was no

"tufting" of the terminal phalanges of the fingers (Plate XXXIX, Fig. 4). The skin was soft and moist. The hair was normal in texture and distribution.

There was an early bitemporal defect in the visual fields (text Fig. 4). Temperature and pulse rates were normal. Urinary output was not increased. The urine contained no sugar. The basal metabolism was −7. Her height was 167 cm., and her weight 57.7 kg.

On November 12, 1924 Dr. Cushing did a transphenoidal operation. When the dura underneath the tumor was incised, soft neoplastic tissue extruded itself in large masses. Additional tissue was removed with the usual instruments.

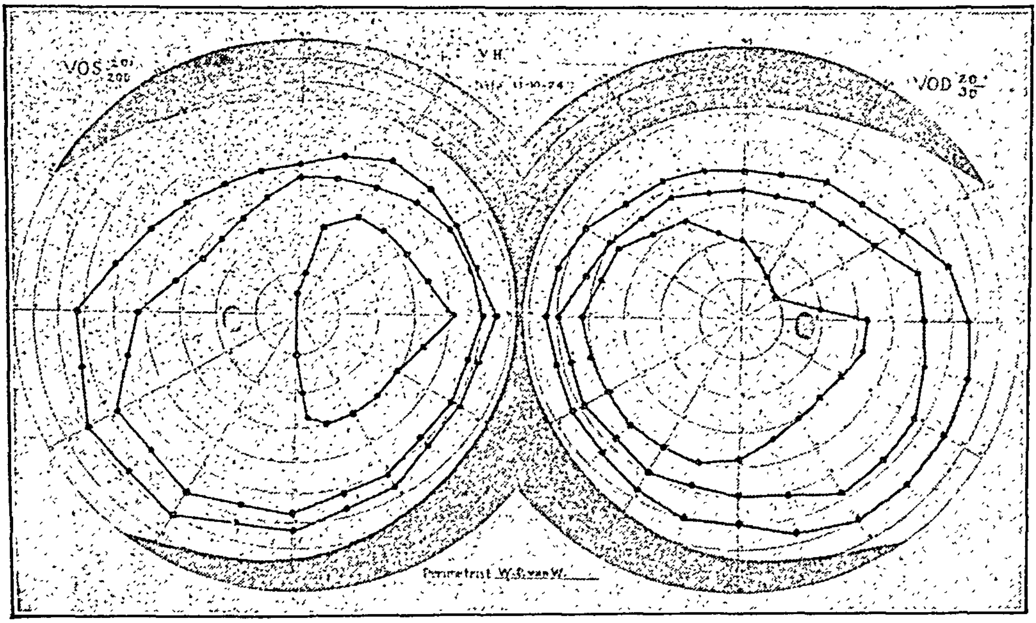

FIG. 4. Visual fields of patient IV.

The patient recovered promptly and was discharged on December 1, 1924. Her visual fields had rounded out normally and visual acuity was much improved.

Histological examination of tissues removed at operation

The tissues were fixed in Regaud's and in Zenker's fluids and were stained with hematoxylin and eosin, neutral ethylviolet-orange G, and with acid violet-eosin.

The tissue is composed of a mass of polygonal cells, with very little connective tissue except that around rare vascular sinuses. Many multinucleated cells are seen. The great majority of the cells contain *a* granules. Many of the cells are so packed with these granules as to appear almost solidly blue (Plate XL, Fig. 4).

SUMMARY OF RESULTS IN ALL CASES

The four cases given in detail are typical of our material, both from the clinical and pathological points of view. The effects of the various fixatives used are clearly illustrated.

The tissues removed from twenty-three patients were fixed in Zenker's fluid or formol-Zenker. This fixation has the defect of agglomerating the α granules and also causes artificial granulation of the cytoplasm. The chromatin granules also stain intensely after this fixation, and the α granules are not differentiated clearly from the background (cf. Plate XL, Fig. 1). The color contrast may be heightened by mordanting the tissue in 3 per cent potassium bichromate for some days before embedding it. Paraffin sections may also be so treated. In this way we have been able to demonstrate numerous α cells in 19 of the 23 cases. In 4 cases we could not find α cells. In 3 of these (Surg. Nos. 4102, 6037, 4430) the cells contained coarse granules, but we were unable to obtain a sufficiently distinct differentiation to be sure they were α granules. However, they were not β granules. Two of these patients were frank acromegalics; the other had a dyspituitary syndrome with some acromegalic features. The fourth tumor in which we could not find granules came from a patient (Surg. No. 10223) who was not diagnosed as acromegaly at the time of operation, but returned later with characteristic changes and died in diabetic coma. Permission for a necropsy could unfortunately not be obtained. The general structure of the tumor in each of these four cases, the loose arrangement of the cells, absence of connective tissue septa, multinucleated cells, etc., made us feel that we were dealing with the same type of adenoma as in the other cases.

In two cases, only formalin-fixed tissues were available for examination. This material is quite suitable, but preparations made from it do not photograph well, as the contrast is not great (cf. Plate XL, Fig. 2). However, in each of these two cases numerous α cells were found. The hypophyses from four necropsies done on acromegalic patients were also examined. They were all fixed in formalin and all contained great numbers of α cells.

There was only one case in which the tissues removed at operation had been fixed in Bensley's fluid alone. This fixation causes granulation of the cytoplasm so that the preparations photographed badly (cf. Plate XL, Fig. 3). Under the microscope the distinction between the artificial granulation of the cytoplasm and the α granules is easier, for the latter are stained blue with neutral ethylviolet, while the former is orange. The contrast, however, is not so sharp as could be wished and the fixative is on the whole quite unsatis-

factory. However, it shows unmistakably a preponderance of α cells. The tissues from the last 9 patients were fixed in Regaud's fluid and in each one great numbers of cells were found so full of granules in many instances that in the microphotograph they appear as a black mass (cf. Plate XXXI, Fig. 2, right).

In contrast to the thirty-five acromegalic hypophyses there are fifty-four hypophysial adenomas taken from patients who showed no signs of acromegaly. In none of them could we find more than a few α cells, scattered through many sections, after a long search. In all but three or four of them no α cells could be found. The general structure of these chromophobe tumors was also different from that of the acromegalic tumors as we have already explained.

Cases from Literature

The following are the cases of acromegaly reported in the literature in which sufficient details of the histological technique are given to enable one to be sure that the technique was adequate for the demonstration of the α granules. We have noted every case in which, in our opinion, the author has shown α granules, although, of course, they were not called by this name.

I. Fränkel, Stadelmann, and Benda,[3] 1901.

Case I. Tumor of hypophysis extending up into third ventricle. Non-granular cells predominated, but many α cells were seen.

Case II. Tumor of hypophysis eroded sellar floor and filled sphenoidal cells. Cells contain fairly coarse α granules.

Case III. Large pituitary tumor — composed almost entirely of α cells.

Case IV. Fairly normal appearing acini around periphery, gradually going over into irregular mass of tumor cells, which are practically uniformly α cells, stuffed with granules. (Presumably toluidin blue-sodium sulphalizerinate used in all cases.)

II. Launois et Roy,[31] 1903.

Large tumor extending into right cerebral hemisphere. Cells of tumor packed closely together are of variable shapes. Many have 2, 3, even 7 nuclei. Amitotic division of nuclei

common. Nearly all cells eosinophile. No cyanophiles. (Presumably used Launois' methylviolet method.)

III. Huchard et Launois,[16] 1903.
Sella turcica 27 mm. × 19 mm. Pituitary small (0.80 gm.); almost lost in the big sella. Necropsy done 2 days post-mortem. Marked connective tissue sclerosis. Authors note delayed post-mortem, and unsatisfactory nature of material. Say most of the cells seem to be cyanophile. (Used presumably Launois' method with methylviolet. Not mentioned in text.) Since this method is a specific stain for α granules, the adenoma was undoubtedly eosinophilic.

IV. Cagnetto,[13a] 1904.
Case II. Hypophysial tumor size of "hen's egg," with metastatic deposit along spinal cord. In the periphery of the tumor fairly well-defined acini. Center of tumor consisted of an irregular mass of cells with no definite arrangement. In the periphery and for a considerable distance into the tumor all the cells were α cells. Fixed in Müller's fluid and used Benda's method and methyl violet. Metastases composed of chromophobe cells.

V. Ballet and Laignel-Lavastine,[26] 1905.
Hypophysis weighed 0.75 gram. There was a great increase in the interalveolar tissue. The glandular cells were almost entirely α cells. No β cells were seen. Iron hematoxylin of Heidenhain.

VI. Lewis,[19] 1905.
Hypophysis said to be macroscopically normal. The component cells very irregularly grouped in the intercapillary spaces with very little connective tissue. A few chromophobe and basophile cells found along boundary between ant. and post. lobe. All the rest were α cells. Some of them contained 2 or 3 nuclei. Used among other methods, iron hematoxylin on formalin-fixed material.

VII. Cagnetto,[13b] 1907.
Case I. Hypophysial tumor weighed 8.55 grams. In the periphery of the tumor typical acini. The center of the growth consists of larger cells not definitely arranged along

vessel walls. Notes many giant cells with 6 or 7 nuclei. States that all the cells of the tumor are chromophobe. Technique not noted, but probably Benda's method and acid fuchsin. Necropsy 30 hours post-mortem.

Case II. Cystic tumor of hypophysis. Necropsy 29 hours post-mortem. Fragments of growth around wall of cyst composed of both *a* and chief cells. Used Benda's method.

VIII. Alquier and Schmiergeld,[32] 1907.

Case I. Hypophysis weighed 8 grams. The hyperplasia composed almost entirely of *a* cells, with large vesicular nuclei. Used iron hematoxylin of Heidenhain. A few basophiles were seen (?) and rarely chromophobe cells. The cells were crowded against each other without any regular disposition. A few blood vessels and almost no connective tissue.

IX. Stumme,[7] 1908. Operative Specimen.

Müller-formol. Used iron hematoxylin, Mallory (?) and Kresofuchsin. Composed of small lymphocyte-like and spindle-shaped cells not arranged in alveoli. Many of them contained numerous fine granules not staining distinctly.

X. Erdheim,[9] 1909.

Tumor in sphenoid sinus. Cells filled with *a* granules. (Heidenhain's iron hematoxylin.) Hypophysis looked normal (19 × 12 × 6 mm.).

XI. Wurmbrand,[33] 1909–10.

Case II. Operative Specimen.

Used iron hematoxylin among other stains. Cells in an irregular mass with practically no struma. Filled with fine *a* granules.

Case III. Operative Specimen.

Used iron hematoxylin among other methods. Mass of cells with no connective tissue stroma. Nuclei vary enormously in size. Many cells with 3–5 nuclei. In the cytoplasm of the cells are numerous *a* granules, finer and more sparsely distributed than in the normal cells.

XII. Lewis,[20] 1910.

Material removed at operation. Fixed in Zenker's fluid and stained with neutral gentian, etc. Material shows enlarged

acini with little interacinous connective tissue. Many of the cells in karyokinesis. Large majority contain α granules.

XIII. Erdheim,[30] 1910.

Case II. Hypophysis measured 1.5 × 1.4 × 0.95 cm. Adenoma (11 × 6 mm.) in pars distalis composed of α cells. Used iron hematoxylin of Heidenhain. The growth was composed of a disorderly mass of cells with large vesicular nuclei. There was very little connective tissue.

XIV. Ballet and Laignel-Lavastine,[11] 1912.

Hypophysis weighed 0.75 gram. There was a great increase in the interalveolar tissue. The glandular cells were almost entirely α cells. No β cells were seen. Stains used for α cells not mentioned, but refer to previous case (V) where they used iron hematoxylin of Heidenhain.

XV. Leotta,[18] 1912.

Tumor of hypophysis 4 × 3 cm. Composed of loose mass of rounded cells with large vesicular nuclei. Few blood vessels. Many cells have more than one nucleus. As many as 7 in a single cell. Most of the cells are eosinophilic. Tissue fixed in 10 per cent formalin and stained with Heidenhain's iron hematoxylin.

XVI. Reinhardt and Creutzfelt,[24] 1913.

Tumor of hypophysis 2 × 3 cm. Very little stroma. Cells loosely arranged with no order. Very fine granules in cells. From 2–8 nuclei seen in a single cell. Used iron hematoxylin and Kraus' hematoxylin.

XVII. Kraus,[17] 1914.

Case 24. Hypophysis a tumor size of a "cherry." Some normal anterior lobe tissue in the periphery. Tumor consists of rounded cells varying in size. Many of them are α cells. The α granules are not so marked as in the normal cells. Very little connective tissue struma and few blood vessels. Evidently used Kraus' hematoxylin.

Case 25. Hypophysis 18 × 20 × 13 mm. A typical eosinophilic adenoma with very little normal anterior or posterior lobe. Kraus' hematoxylin used.

Analysis of the Literature

In only two cases, to our knowledge, where presumably adequate technique was used, has the tumor been stated not to be an eosinophilic adenoma. The first was Cagnetto's Case I.[13b] We need only indicate that the necropsy was done 30 hours post-mortem, giving ample time for the delicate granulation to be lost. No such objection can be brought against Stumme.[7] His material was removed at operation and presumably fixed at once. It was fixed in Müller's fluid and properly stained. His observation is, therefore, probably correct that the cells contained a fine granulation which stained poorly by methods for α granules. Such a granulation we have seen in the case of a fat patient with dyspituitrism and some acromegalic features. It is unfortunate that Stumme gives sketches of his patient instead of photographs, for in sketches interesting details, such as texture of skin, etc., cannot be judged.

Cagnetto, when he thought he had found a case of acromegaly in which the tumor was not eosinophilic, stated that now there was needed, to overthrow the hyperpituitary theory of acromegaly, only a case of tumor in which the cells contained α granules but the patient was not acromegalic. Such a case he thought was supplied by Zak.[8] But Zak, who evidently is familiar with the literature, and gives a very judicious estimate of his case of pituitary tumor without acromegaly, makes no claim to have discovered an eosinophilic adenoma here. He simply states that "the demonstration of Benda's secretory granules, because of the unfavorable fixative (alcohol) for this purpose, met with only indifferent success."

It is repeatedly noted in the above reports that many of the cells in the hypophysis of acromegaly contain several nuclei. The frequence of amitotic division is also noted. These phenomena have been long known and were seen by Osborne (1922),[22] Neal and Shattuck (1898),[21] Brooks (1898),[34] Dalton (1898),[15] Claude and Baudouin (1911),[14] Meyer (1913),[39] Ausch (1918),[10] and many others.

In three patients often quoted in the literature as acromegalic[38] the pituitary body is said to have been normal (Bonardi,[4] Petren,[23] Yamada[35]). In the cases of Petren and Yamada we are willing to admit that the hypophysis was normal, but question whether either patient was acromegalic. The hypophysis from Bonardi's patient

was four times normal weight (2.975 gms.), and details of the histological examination given seem to indicate that it was not adequate, a fatal error in the absence of a tumor, as the cases of Lewis and of Huchard and Launois demonstrate. Besides, as a case of Erdheim (X) shows, when the pituitary body is of normal size, a search must be made of the sphenoid region and pharynx.

We must, finally, mention the often quoted report of Bleibtreu [6] in which a hemorrhagic cyst was found in the hypophysis of a giant acromegalic. There is no evidence whatever from the history to determine when the hemorrhage took place, and we feel no hesitation in affirming that it occurred in a soft adenoma, after the acromegalic syndrome was established. Such a hemorrhage we have ourselves observed.

It is obvious, therefore, that, in the light of our own experience and after a careful scrutiny of the literature, we believe that satisfactory evidence does not exist of the presence of acromegaly without an increase in the number of the eosinophilic cells of the pars distalis of the hypophysis cerebri.

Conclusion

In view of the foregoing description of histological material and analysis of the literature, we feel justified in concluding that, in those cases of acromegaly at least in which the gland reaches a size sufficient to demand operation, a constant pathological change takes place in the pars distalis of the hypophysis cerebri, consisting essentially of an adenomatous formation composed of α cells. The normal acinous structure tends to be lost; the cells lie in a loose mass, practically without stroma; the nuclei vary greatly in size and as many as half a dozen may be present in one cell; the cells contain α granules which are finer than the normal α granules and which tend to collect in the periphery of the cytoplasm.

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DESCRIPTIONS OF PLATES XXXI–XL

Plate XXXI

Fig. 1. Microphotographs of normal hypophysis cerebri.
left. Hematoxylin and eosin stain. x 300.
right. Acid fuchsin-acid violet stain. x 850.
a. α cells. b. β cells.
c. chromophobe cells.

Fig. 2. Microphotographs of chromophile adenoma from a patient with acromegaly.
left. Hematoxylin and eosin stain. x 300.
Note absence of connective tissue and of blood vessels; also multinucleated cells.
right. Neutral ethylviolet-orange G stain. x 850.
Cells are packed with α granules, which alone appear black in photograph.

Fig. 3. Microphotographs of chromophobe adenoma of hypophysis from a patient with hypopituitary syndrome.
left. Hematoxylin and eosin stain. x 300.
Note the elongated epithelial cells in large masses, separated by thin connective tissue septa carrying small blood vessels.
right. Phosphotungstic acid-hematoxylin stain for mitochondria. x 850. The fine granular mitochondria are so numerous that they outline well the boundaries of the cells.
No other granules than mitochondria exist. These may persist in the cells and be mistaken for α granules.

Plate XXXII

Fig. 1. Microphotograph of hypophysial adenoma from a patient with acromegaly.
Fixed in Zenker's fluid.
Hematoxylin and eosin stain. x 850.
Note multinucleated cells and crescent formation.

Fig. 2. Microphotograph of hypophysial adenoma from a patient with a hypopituitary syndrome.
Fixed in Zenker's fluid.
Hematoxylin and eosin stain. x 850.
Note elongated cells along the septa and the indistinct cell boundaries.

Plate XXXIII

Case I. Note the prognathism and prominent frontal sinus.

Plate XXXIV

Case II. Note the adiposity, coarsening of features and extremities, and absence of prognathism.

Plate XXXV

Fig. 1. Lateral roentgenograph of sella of Case I. Nat. size.

Fig. 2. Lateral roentgenograph of sella of Case II. Nat. size.

Plate XXXVI

Case III. Note prognathism and prominent frontal sinus.

Plate XXXVII

Case IV. Note slight coarseness of nose and lips and absence of prognathism, also thick, square fingers.

Plate XXXVIII

Fig. 1. Lateral roentgenograph of sella of Case III. Nat. size.

Fig. 2. Lateral roentgenograph of sella of Case IV. Nat. size.

Plate XXXIX

Roentgenographs of middle fingers of Cases I, II, III, IV.
Note width of fingers, and tufting of terminal phalanges in every case except the last.

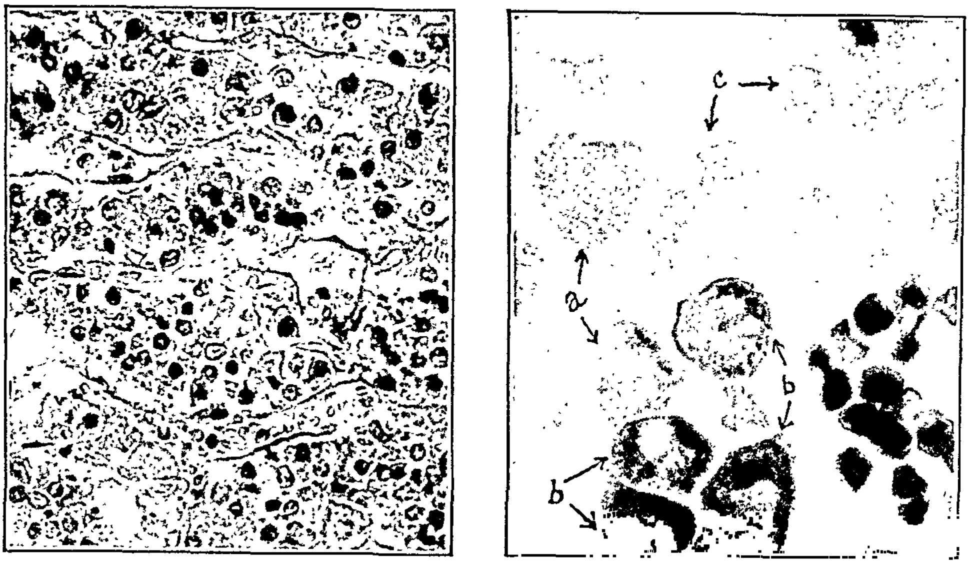
Plate XL

Fig. 1. Microphotograph of tumor from Case I. Zenker fixation.
Neutral ethylviolet-orange G stain. x 850.
Note that the nuclei are well stained but α granules not well differentiated.

Fig. 2. Microphotograph of tumor from Case II. Formalin fixation.
Neutral ethylviolet-orange G stain. x 850.
Note that the α granules are well differentiated but contrast is not great.

Fig. 3. Microphotograph of tumor from Case III. Bensley fixation. Neutral ethylviolet-orange G stain. x 850. Note the artificial granulation of the protoplasm due to the fixative. Under the microscope the α granules may be distinguished by their blue color. The differentiation is not so distinct as it is on Regaud-fixed material.

Fig. 4. Microphotograph of tumor from Case IV. Regaud fixation. Neutral ethylviolet-orange G stain. x 850. Only the α granules and erythrocytes appear black in photograph.



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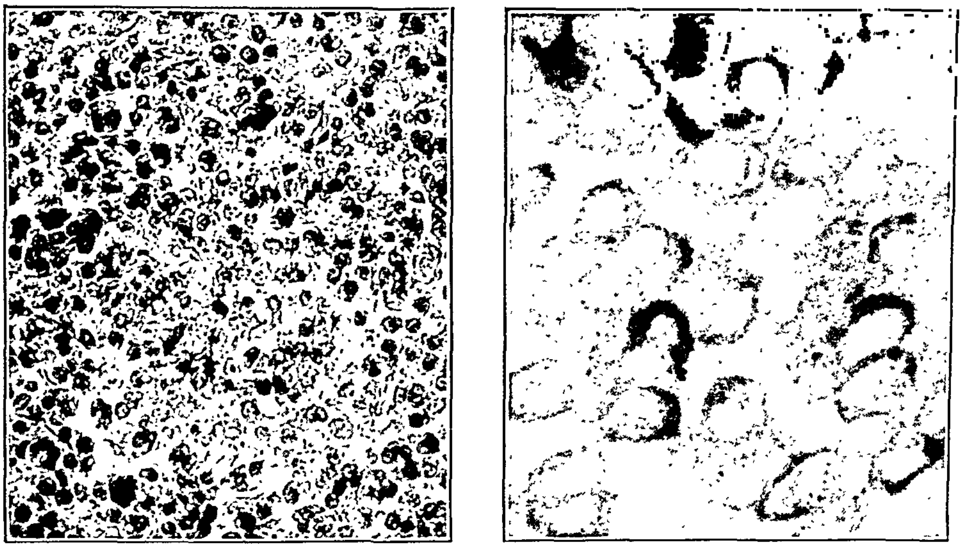

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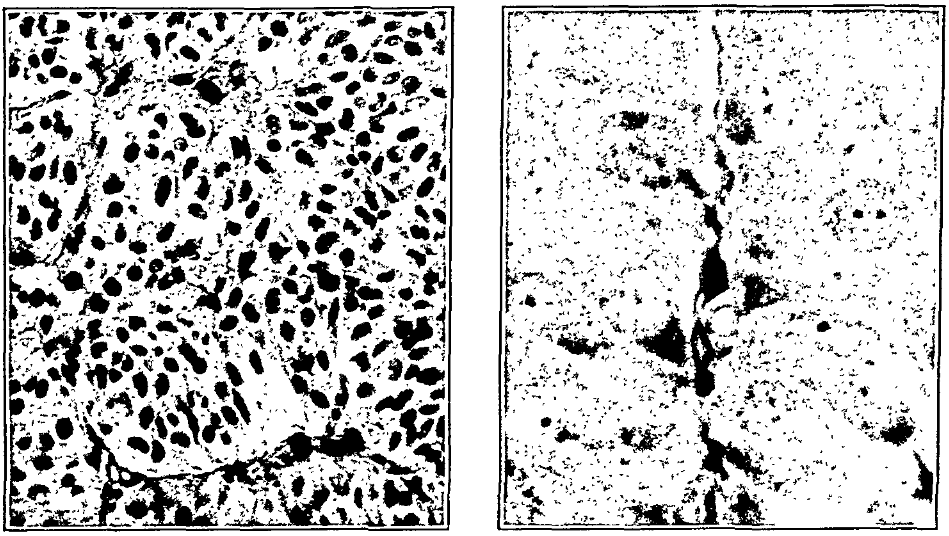

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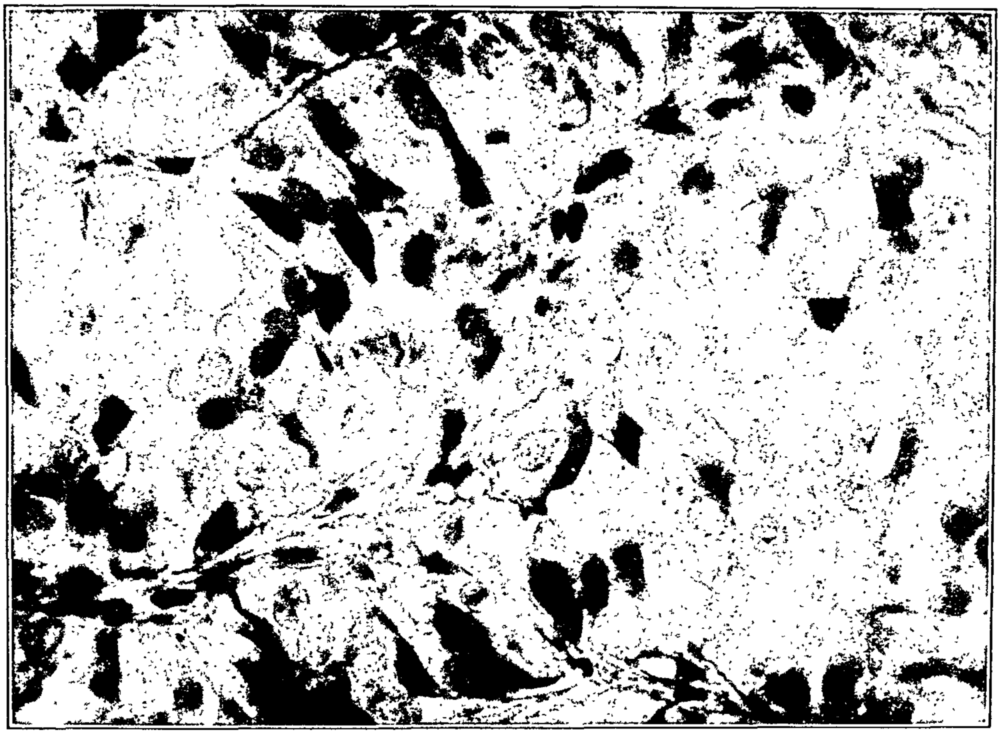

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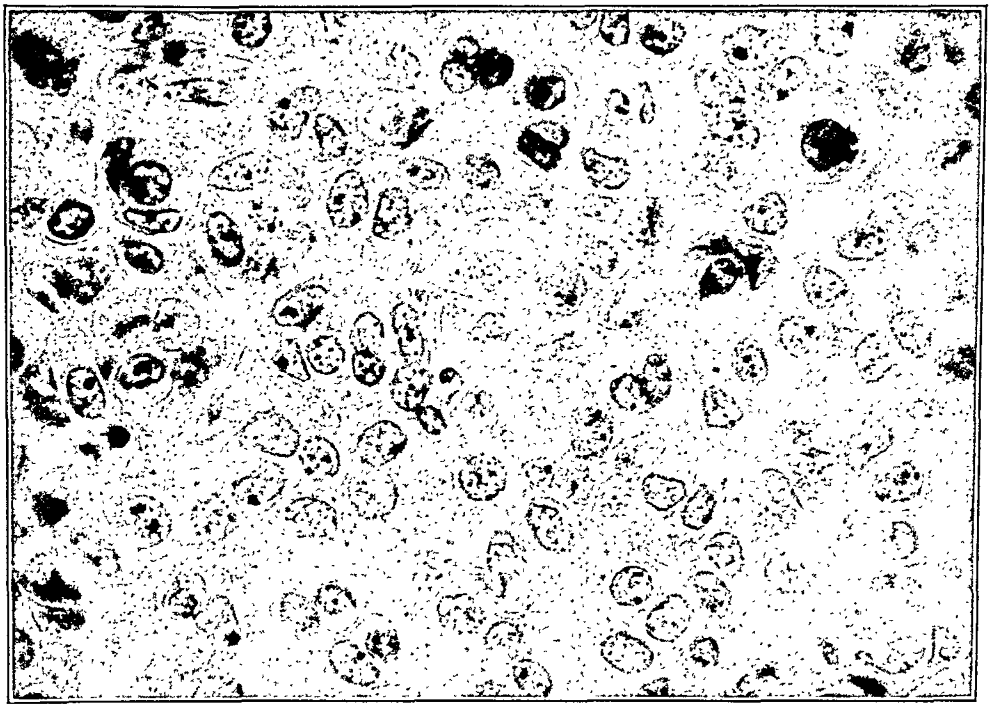

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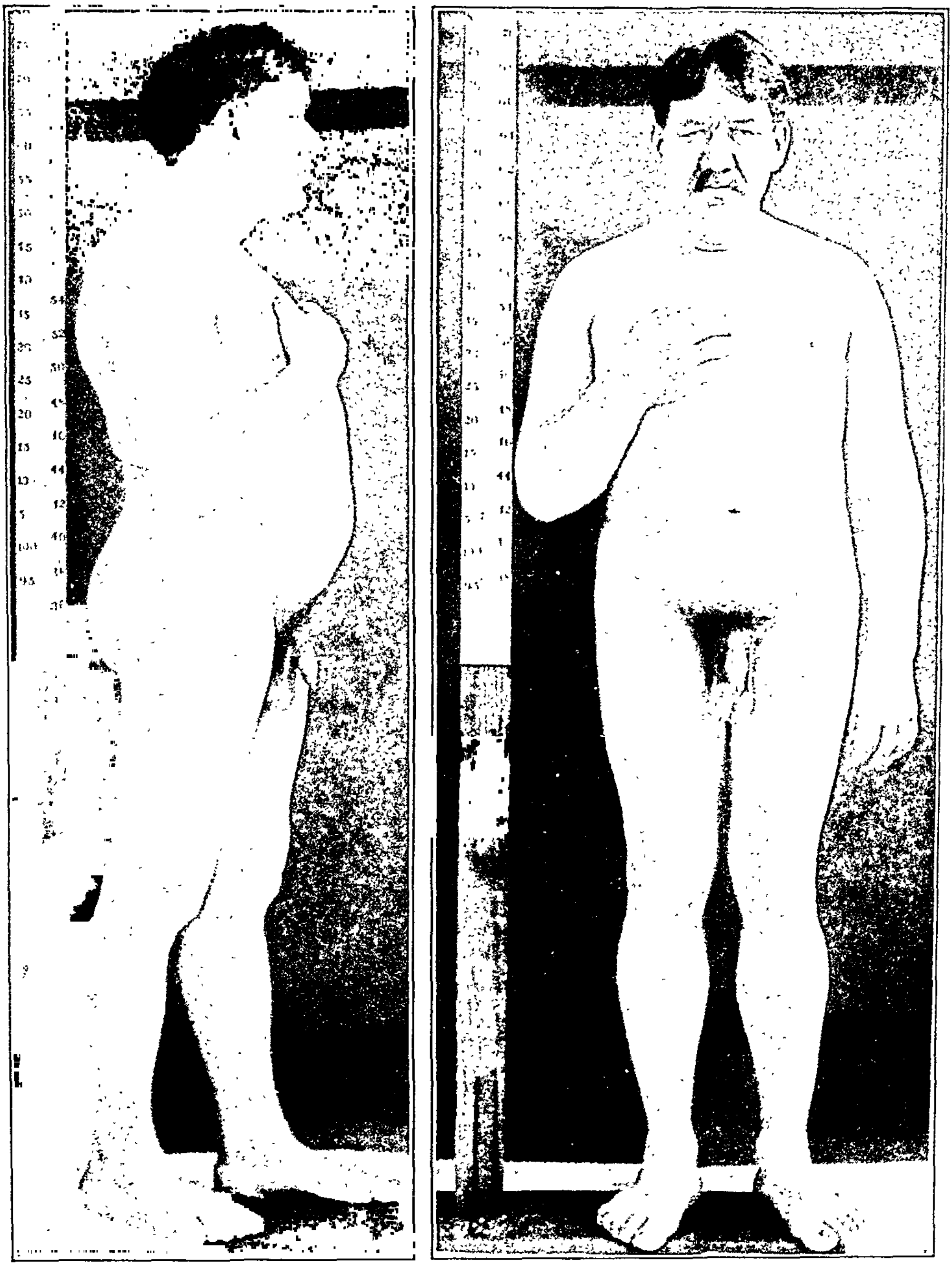
Bailey and Davidoff Hypophysis cerebri in acromegaly

Bailey and Davidoff Hypophysis cerebri in acromegaly



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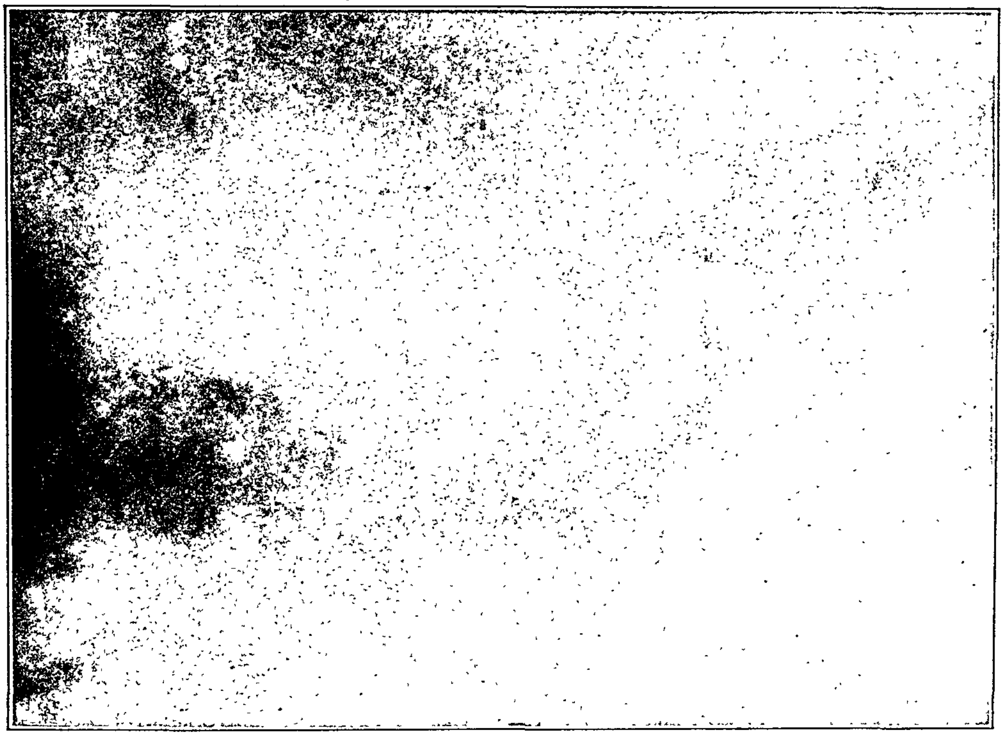

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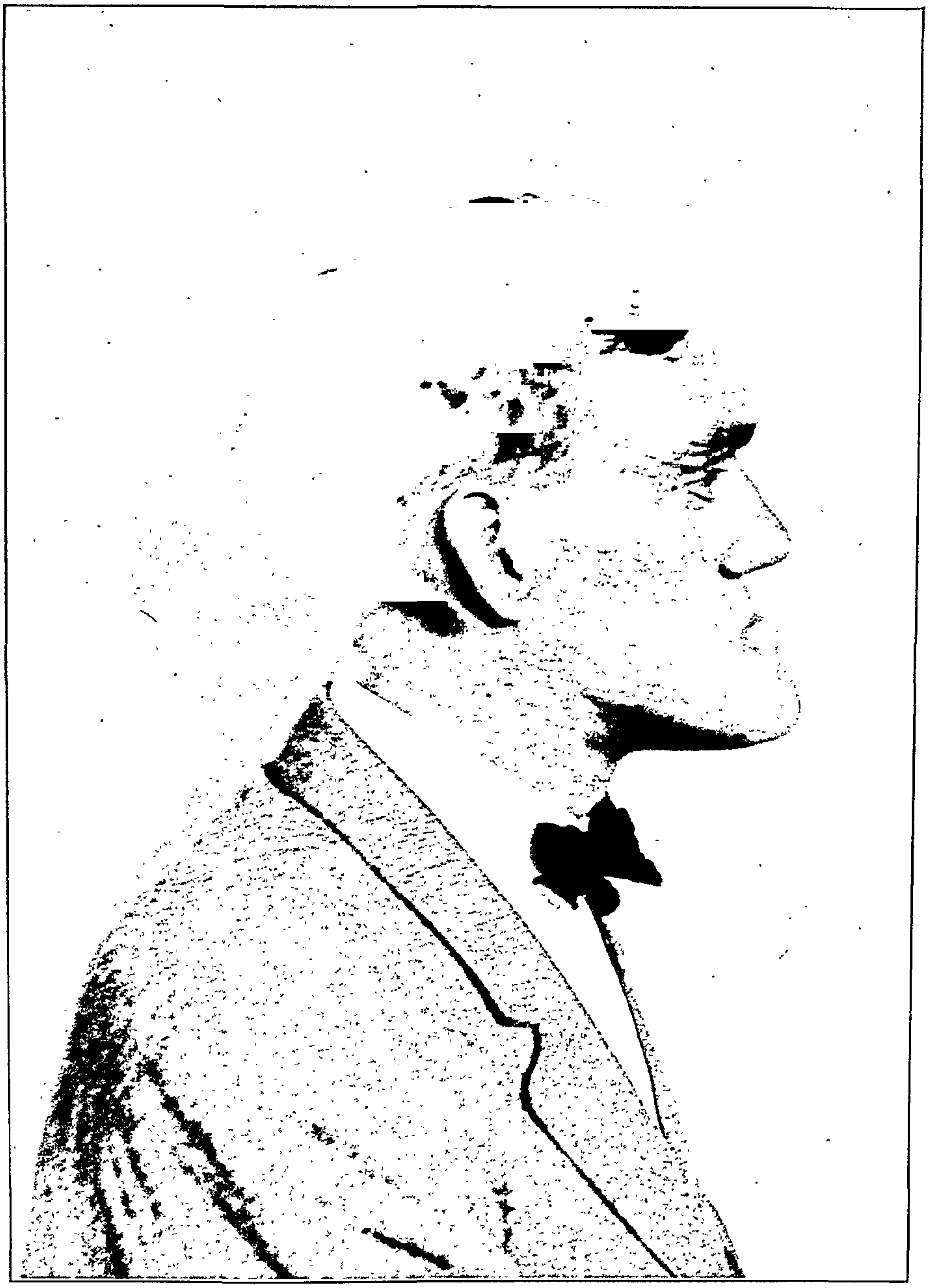
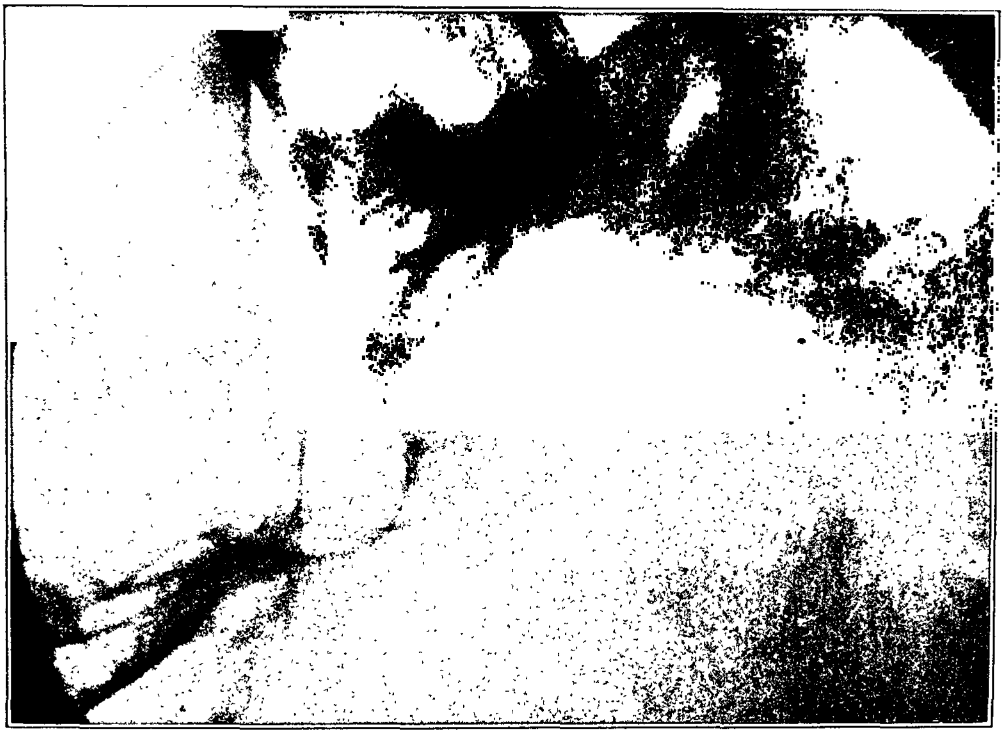


Bailey and Davidoff Hypophysis cerebri in acromegaly

Bailey and Davidoff Hypophysis cerebri in acromegaly



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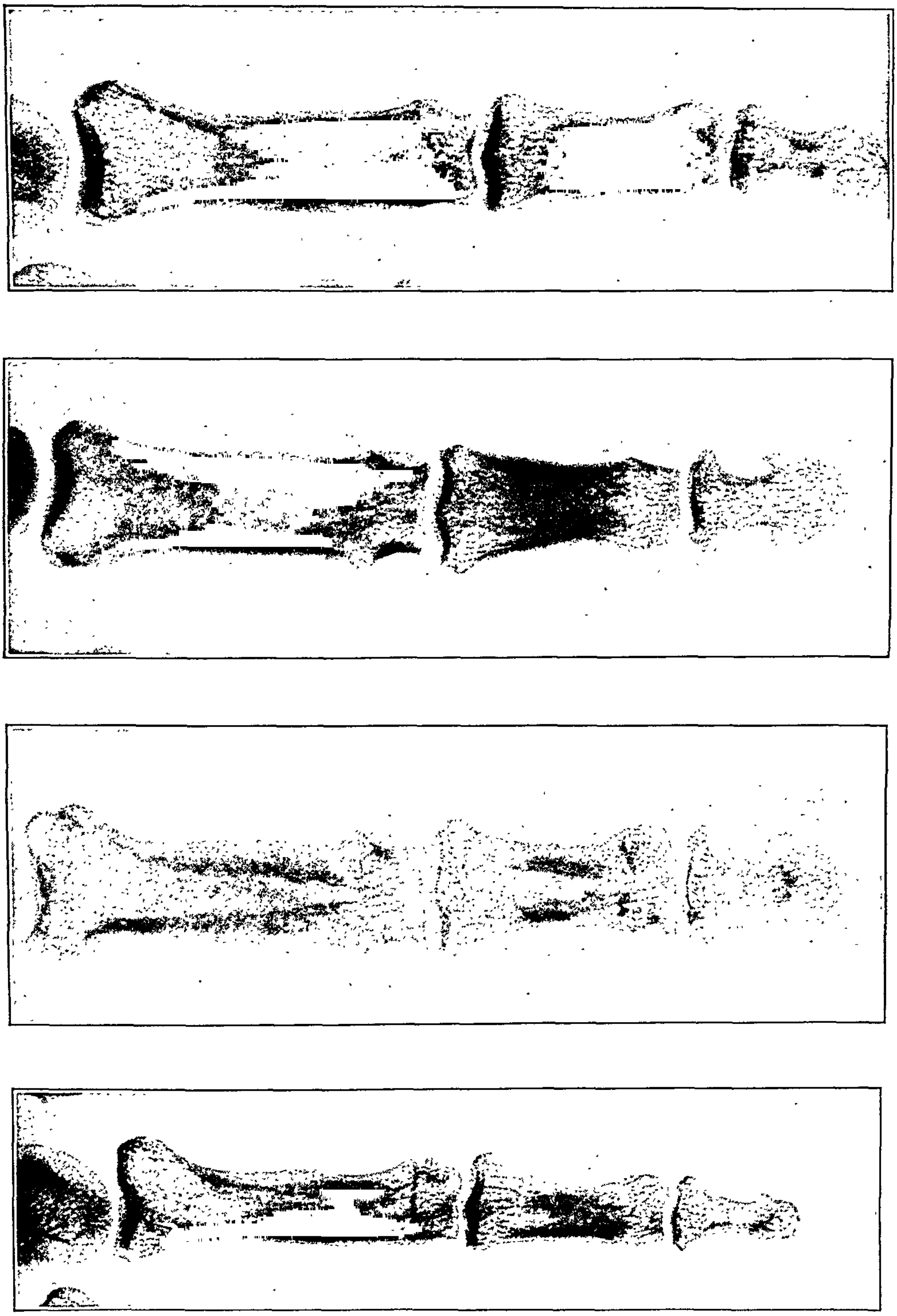

Bailey and Davidoff Hypophysis cerebri in acromegaly

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Bailey and Davidoff

Hypophysis cerebri in acromegaly

THE PATHOLOGY OF THE HYPOPHYSIS *

I. THE PRESENCE OF ABNORMAL CELLS IN THE POSTERIOR LOBE

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The deficiency of accurate knowledge as to the function or functions of the hypophysis, and the effect on the general economy of the body of pathologic processes in this organ, render it desirable to seek information concerning its pathology from all possible sources. This small gland is all too frequently neglected in the usual post-mortem examinations even when the head is opened and the brain removed. It has been our practice for some years to examine in serial sections all hypophyses removed at necropsies. As a result of these routine examinations a number of distinct abnormalities have been observed. It is proposed in a series of papers, of which this is the first, (1) to describe the various lesions encountered; (2) to search in the associated pathology for some clew as to the etiology of the pathologic process in the hypophysis; and (3) to make a careful analysis of the clinical histories for possible clinical manifestations of the lesion.

In 1922, one of us[1] described the presence of a group of abnormal cells in the posterior lobe of the hypophysis. Since that time, eight similar cases have come under our observation. Two papers dealing with a like condition have appeared in the literature.

Sternberg[2] has described in the neurohypophysis a group of abnormal cells which he designated as a Choristoma. The patient was a man 65 years old. The outstanding clinical manifestations were fatigue on slight exertion, without dyspnoea or palpitation; marked anemia without emaciation; a generalized persistent and obstinate edema that was neither cardiac nor renal in origin; and a marked retention of water and sodium chloride. At necropsy a large medullary carcinoma of the stomach was found. The heart, kidneys, thyroid, lungs, liver and brain showed no gross pathologic changes. The hypophysis was not enlarged. On surfaces made by sectioning there was seen in the posterior lobe a very small white

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mass, distinctly visible against the general grayish brown color of the lobe. On microscopic examination this mass was found to be composed of cells, in all essentials of morphology and arrangement, similar to those shown in Figure 1 of our series.

In 1922, Priesel[3] reported 29 cases in which he found very small masses of misplaced tissue in the posterior lobe of the hypophysis. In some five of his cases the cells were distinctly more spindle-shaped and were not, therefore, in all respects similar to the instances reported by one of us and by Sternberg. Very scant clinical data were given by Priesel. None of his patients showed edema that could not be readily accounted for either on a cardiopathic or a nephropathic basis.

The groups of abnormal cells in our cases varied somewhat in size, but were essentially similar in morphology and arrangement. A general description of these masses of cells will be given after briefly summarizing the other features of the cases.

Case 1. Colored male. Estimated age, 65 years. Found dead (murdered) in a basement. No clinical history was obtainable. He was six feet, two inches tall and "rawboned" but not emaciated; the hands and feet were quite large; prognathism was not present. There was no abnormal growth of hair on the body. Adrenals, thyroid and and testicles showed no important gross pathologic changes, and were of approximately normal size. The hypophysis weighed 0.65 gram. In the posterio-lateral quadrant of the neurohypophysis, about 0.5 mm. from the median line, was a mass of abnormal cells measuring approximately 0.75 mm. in diameter. Cells from the middle lobe had grown backward into the posterior lobe; but these were entirely different in character from those composing the cellular mass, and serial sections showed no connection between them. In addition, a small chief cell adenoma, 3.5 mm. in diameter, was found in the anterior lobe.

Case 2. Colored male, aged 35 years. No reliable clinical data obtainable. Death was due to cerebral hemorrhage. The thymus was atrophied but small masses of thymic tissue were present in the fat behind the upper end of the sternum. Adrenals and thyroid apparently normal. The hypophysis was not enlarged. In the lateral and posterior part of the neurohypophysis was a mass, 0.25 mm. in diameter, composed of abnormal cells similar to those in the

other cases of this series. There was a slight backward growth of cells of the middle lobe into the neurohypophysis which had no connection with the mass of abnormal cells.

Case 3. Well nourished and well developed colored male, 32 years old. The chief symptoms complained of were shortness of breath on exertion and edema which became more and more marked until death. Necropsy revealed an insufficiency of the aortic valve due to malformation of one of its cusps, believed to have been congenital. Adrenals and thyroid normal in appearance. Approximately in the median plane of the posterior lobe of the hypophysis, was a mass of abnormal cells, the whole measuring 0.25 mm. in diameter. This mass differed from the others in this series only in that it was U-shaped and formed a sort of cap over a vascular loop made by an artery and vein.

Case 4. White female, aged 47 years. Markedly emaciated, and had the appearance of a woman 65 years old. She "drank a large amount of water," but no definite information was available as to the excretion of water and sodium chloride. She had syphilitic ulcers of the foot and tabes dorsalis. Wassermann test strongly positive. At necropsy all of the organs were more or less atrophied. In the posterior lobe of the hypophysis were three small groups of cells, somewhat smaller than those in the other cases here reported. These cells were embedded in a finely granular material which occupied the center of a small cavity; they had distinct outlines; their cytoplasm was clear and non-granular; their nuclei were large and stained rather palely. From the embedding material finely branching processes extended toward the walls of the cavities, which were lined with plump spindle-shaped cells. No blood vessels could be seen within the masses of cells.

Case 5. Well developed and well nourished colored man, 54 years of age. No note as to the quantity of urine passed. No edema. Death was due to lobar pneumonia complicated by a pneumococcic meningitis. The adrenals and thyroid showed no gross or microscopic pathologic changes. The testicles were atrophied and markedly pigmented. The hypophysis was not increased in size. In the postero-lateral quadrant of its posterior lobe was a millimeter-sized mass of cells with the morphology common to this group of cases. These cells were arranged in cords which ran more or less parallel

with each other and were separated by a fine supporting tissue. No vessels containing blood could be distinguished between the cords of cells.

Case 6. White male, aged 66 years. Clinical diagnosis, paralysis of the lower extremities due to pressure on the spinal cord by a tumor of the vertebrae. Well nourished but not obese. No edema. Quantity of urine ranged from 300 c.c. to 1125 c.c. per day; later developed incontinence and on some days as little as 60 c.c. of urine were collected. Nearly all of the specimens of urine contained a small amount of albumin and a few pus cells but no casts. Wassermann test negative. Necropsy showed a myeloma of the left 6th rib and of the 6th thoracic vertebra; hypostatic pneumonia; marked generalized arteriosclerosis; arteriosclerotic contracted kidneys. The adrenals showed no gross changes. The right lobe of the thyroid was enlarged and contained a mass which was diagnosed a tumor of the parathyroid. The testicles showed no gross abnormalities. The hypophysis was of normal size. In a postero-lateral quadrant of the neurohypophysis was a group of abnormal cells measuring 0.5 mm. in diameter. In addition this hypophysis showed a very marked fibrosis of the anterior lobe and a moderate backward growth of cells from the middle lobe into the neurohypophysis.

Case 7. White male, aged 52 years. Cause of death, influenza with bilateral bronchopneumonia. The hypophysis was of normal size. In a postero-lateral quadrant of its posterior lobe was a mass, 1 mm. in diameter, composed of large cells which tended to be arranged in long cords. A considerable amount of brownish pigment was present in this neurohypophysis, but none within the mass of cells.

Case 8. No clinical or other data available. At about the center of the posterior lobe of the hypophysis was a group of less than a dozen cells similar to those in the other cases of this series.

All of these patients showed in the posterior lobe of the hypophysis a fairly well circumscribed cellular mass, not encapsulated, and varying in size from less than 0.25 mm. to slightly more than 1 mm. in diameter. In all except one case this mass of cells was in one postero-lateral quadrant of the posterior lobe. They were not so near the median plane of the lobe as Priesel states was the case in his specimens.

These masses are composed of large, round, oval or polygonal cells which stain a bluish-purple and have abundant granular cytoplasm. The nuclei are round and usually eccentrically placed. In many cells they are deeply stained and pyknotic, or pale and apparently undergoing karyolysis. Some of the cells are entirely devoid of nuclei; others show shadowy nuclear remains. No nucleoli can be seen in any of the cells. Within the masses, the cells frequently occur in groups of 2 to 6 in direct contact with each other. The supporting tissue of these masses is scanty in amount and resembles that of the remainder of the posterior lobe, being characterized by an interlacing meshwork of very fine fibrils. In every case, except Case 4, many of the large cells give off from their peripheries, fine processes that take part in the formation of the intercellular fibrillar meshwork. Among these fibrils there are smaller cells with round and spindle-shaped nuclei. In a few places between the large cells of these masses, there are narrow spaces lined with long spindle cells with slender nuclei. Occasionally one or two red blood cells can be seen in these spaces. But the total quantity of blood in the vessels within these masses is very small. Pigment is absent from these masses of cells.

Two problems present themselves in connection with the lesion here described: (1) What effect, if any, has it on the function of the hypophysis; and (2) what is the nature of the mass and the origin of the cells that compose it?

1. Rasmussen [4] found that the posterior lobe of the hypophysis represents 18 per cent of the total organ. The average hypophysis weighs about 0.6 gram, and measures 13 × 10 × 6 mm. It is evident, therefore, that a mass of abnormal cells even so small as 0.25 mm. in diameter occupies a relatively considerable portion of the neurohypophysis. It would seem quite possible that such a mass, especially one of the larger ones, might produce some change in the function of the structure in which it occurs. The lesion may, therefore, have more than a mere theoretical interest.

Sternberg [2] believed that there was a definite causal relation between the lesion in the posterior lobe of the hypophysis and the marked retention of water and sodium chloride which was apparently the cause of the edema in his patient. Priesel [3] found no such association of retention and edema with the hypophyseal lesion in his cases. In our series, the only instance of edema was clearly

of cardiopathic origin. One patient (Case 6) did, apparently, eliminate relatively little water, but there was no edema, and no accurate information as to the intake of water. In none of our cases was there any record of excessive thirst and nothing resembling diabetes insipidus. While the clinical histories in some of our cases were unavoidably incomplete, none of the functional or bodily changes usually ascribed to lesions of the hypophysis was observed in any of our series. Nor was there encountered any structural or functional abnormality common to a sufficient number of these cases to warrant attributing it to the lesion here described. It is not impossible, however, that a more careful study of patients with this lesion in mind, may reveal some clinical manifestation ascribable to it.

2. Sternberg [2] and Priesel [3] believe that the masses of abnormal cells found in the posterior lobe of the hypophysis were definitely neoplastic in nature. The former classed the "growth" as a Choristoma; the latter as a "Progonoblastoma," a term used by Mathias [5] to designate a tumor which originates in "atavistic formations," "a developmental reversion in a phylogenetic zone of proliferation of an organ in some particular part of the body."

If this lesion is a true neoplasm, the origin of the cells which compose it is not easily determined. The neurohypophysis is derived from the central nervous system. It is composed, except for blood vessels and the scant accompanying connective tissue, chiefly if not wholly of glia cells. The presence of ganglion cells in this structure has been disputed. Benda [6] mentions cells which "resemble sympathetic ganglion cells." Neubert [7] described "pigmented, calcified and glycogen-containing ganglion cells" in the posterior lobe of the hypophysis. Kohn [8] has "never encountered ganglion cells in the hypophysis in man."

Sternberg [2] believes that the lesion is "a blastomatous growth of (ausgereifter) neurogenic elements," probably of glia cell origin. Cells somewhat similar to those described in these lesions have been observed in gliomas. They have been described as "giant glia cells" by Meyer,[9] "monster glia cells" by Storch,[10] and ganglion cells by Robertson.[11] Ribbert [12] has described a "spongioblastoma" of the brain in which there were large cells resembling in many respects those in the lesions here reported. (See Fig. 15, in Ribbert's article.) He believes that these "spongioblasts" are glia cells that have reverted to an earlier embryonic stage than is usually observed in

gliomas. Kohn [8] in Fig. 1 of Plate XV of his article pictures cells that are apparently identical in morphology with those in our cases, and classes them as "very large glia cells rich in protoplasm."

Priesel [3] believes that these "growths" originated from remnants of the cells lining the central tube of the infundibulum and were, therefore, of ependymal origin. His conclusion appears to have been based upon the fact that, in his cases, the masses of abnormal cells were in or near the sagittal plane of the neurohypophysis. In the majority of our cases the cell masses were situated in one of the posterolateral quadrants.

A further possible origin of these abnormal cells is from cells which are frequently found growing backward into the neurohypophysis from the middle lobe of the gland. This is unlikely (1) because the cells which compose these lesions are wholly unlike those which grow backward from the middle lobe; and (2) because serial sections fail to reveal any connection whatever between them.

Sternberg's patient was 65 years old. Of Priesel's cases, all but 2 were over 50 years old, and all but 5, over 60. The patients whose hypophyses we have examined in serial sections (over 200 in number) were of all ages, from the newly born to persons over 70 years old. Among these were the 8 cases here reported. Two of these patients were 32 and 35 years old respectively; one was 47, but looked to be 65; two were over 50, and two over 60 years old. It appears probable, therefore, that this lesion is more common in the later decades of life.

It is our opinion that the large cells in this lesion are changed glia cells, because many of them give off from their surfaces fine branching processes which take part in the formation of the intercellular fibrillar meshwork. Some of the cells in these lesions are undergoing degeneration or even necrosis, as is shown by the character of the cytoplasm and the nuclei described above. We cannot agree with Priesel that this lesion is a tumor originating in remains of the epithelium lining the central tube of the infundibulum, (1) because none of our cases showed columnar or cuboidal cells forming more or less well defined lumina, characteristic of ependymgliomas; and (2) because the cell masses in our cases were located well away from the median plane of the posterior lobe of the hypophysis.

Without being dogmatic, we are inclined to consider the lesion here described as a localized retrogressive change in the glia cells, the cause of which is not now apparent.

Summary

1. A peculiar lesion consisting of a group of large abnormal cells in the posterior lobe of the hypophysis is described.

2. This mass of cells, although actually small, is relatively large when compared with the size of the posterior lobe itself.

3. The nature of the lesion is not clear. It is probably not a neoplasm.

4. Nothing in the clinical histories or physical findings in any of our cases could be definitely associated etiologically with this lesion. Careful study of patients with this pathologic process in mind may, however, reveal some clinical manifestation ascribable to it.

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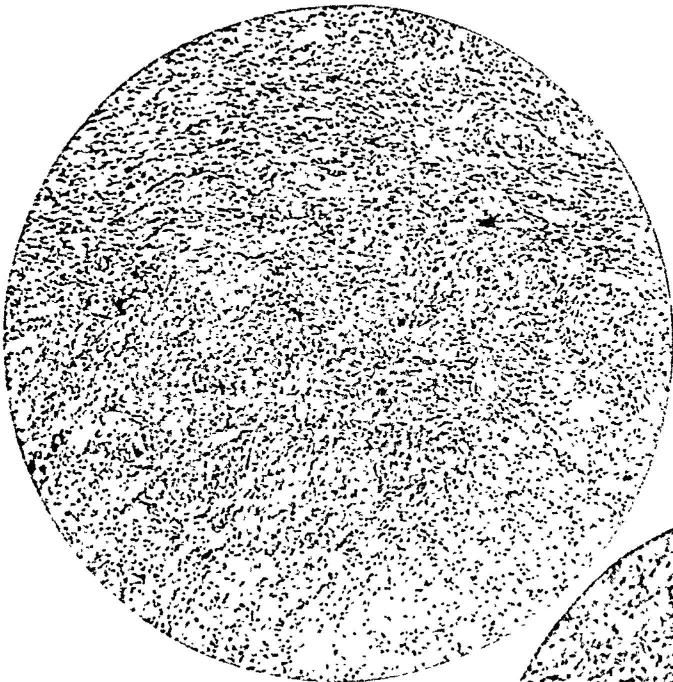
DESCRIPTION OF PLATE XLI

Fig. 1. From Case 7. One of the larger masses. x 45.
Fig. 2. From Case 1. x 82.
Fig. 3. From Case 3. Showing the relation of the mass of cells to blood vessels in this case. x 112.



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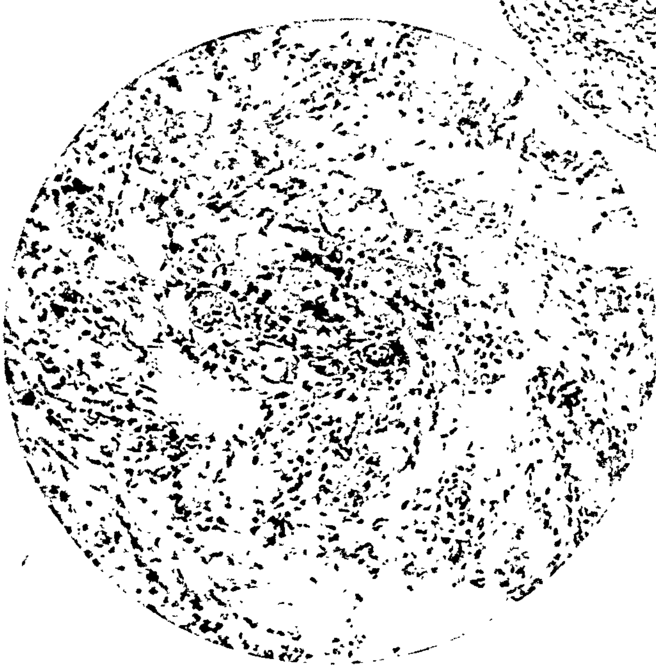

3

Simonds and Brandes

The pathology of the hypophysis

THE HYPERTROPHY OF THE SUBMAXILLARY GLAND IN THE ALBINO RAT, FOLLOWING PARATHYROIDECTOMY *

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The present report deals with the microscopic examination of the enlarged submaxillary glands of the parathyroidectomized albino rat. Hammett (1922) found that removal of the parathyroid glands from this animal resulted in a marked and valid increase in the size

Table 1

Nuclear counts in control (C) and parathyroidectomized (E) animals.

C	E	C	E	C	E	C	E	C	E	C	E	C	E
22	10	19	7	16	8	20	10	11	12	8	9	12	10
24	9	19	8	12	15	15	13	11	10	15	15	16	12
15	16	22	8	19	12	17	10	13	5	16	10	15	15
20	7	16	13	24	10	24	12	17	15	14	11	17	12
15	15	22	14	16	7	18	13	18	5	13	10	12	13
23	11	16	16	22	14	20	10	13	6	11	10	16	10
19	6	21	11	11	10	19	14	20	14	17	8	15	6
26	9	17	17	10	15	18	14	16	12	16	9	23	7
15	15	15	15	16	12	18	13	16	6	18	5	20	9
18	14	16	18	19	12	20	15	11	11	20	13	15	7
Average: 19.7	11.2	18.3	12.7	16.5	11.5	18.9	12.4	14.6	9.6	14.8	10.0	16.1	10.1

Average for controls, per unit field 16.98
Average for parathyroidectomized, per unit field 11.07

of the submaxillary glands. This result did not follow thyro-parathyroidectomy. As appears from Hammett's Table 1, the weight of the glands of the parathyroidectomized animal may exceed that of the litter control by as much as 100 per cent. An increase in weight is however not necessary. A decrease of as much as 21.2 per cent is recorded. In a later paper, Hammett (1923) presented

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evidence that the growth of the submaxillary glands of both sexes was increased over the normal by the lack of parathyroid secretion,

Fig. 1. Natural size. The larger submaxillary gland came from the parathyroïdectomized animal: the smaller from the litter, sex and age control. The nuclear counts given in Table I were made from sections of these glands.

and noted that this effect was much more marked in the females. As an example may be cited a typical case where the glands came from two females of the same litter, killed at the same time. The larger gland weighed 0.7562 gr. and came from a parathyroidectomized animal weighing 167 grams. The smaller weighed 0.4240 gr. and came from the control weighing 217 grams. The larger gland weighed over 178 per cent of the smaller.

TABLE 2

Nuclear counts in control (C) and in thyro-parathyroidectomized (E) rats.

	C	C	C	E	E	E
	18	19	16	21	19	17
	18	19	15	22	23	13
	11	12	18	18	19	19
	12	17	15	16	22	23
	16	17	14	14	16	20
	14	14	16	16	18	15
	13	14	15	17	17	19
	15	11	11	15	18	16
	15	14	15	16	20	17
	22	21	20	14	23	15
Average	15.4	15.8	15.5	16.9	19.5	17.4

Average for controls, per unit field 15.56
Average for thyro-parathyroidectomized, per unit field 17.93

Material and methods. The submaxillary glands used in this study were very kindly furnished by Dr. Hammett. They were fixed in Bouin's or Zenker's fluid immediately after removal and

weighing. For each pair of glands from an experimental animal there was also taken for control a pair of glands from an animal of the same litter, sex and age. Glands from sixteen animals were prepared and studied: 6 parathyroidectomized, 3 thyro-parathyroidectomized, and 7 controls. In two instances the same control served for both types of experimental animals. The tissues were embedded in paraffin and cut serially at 5 μ. In some instances the plane of section was parallel to the cross-section of greatest area: and in other instances, parallel to the cross-section of least area. In mounting the sections, for economy, four or five contiguous sections were spread on the slide and then the next four or five discarded, and so on until all were spread. The "discarded" sections were usually employed for special stains for mucin and connective tissue.

In some instances sections from the gland of a parathyroidectomized animal and from that of the corresponding control, cut in similar planes, were mounted on the same slide. This of course ensured the same conditions of staining. The counts of nuclei recorded below were made from such slides.

The Submaxillary Gland of the Rat

Ranvier[13, 14] and Loewenthal[8] have described the normal structure of this organ in the rat. At the cephalomesial aspect on the ventral surface a well-defined lobe is recognizable lying in a depression in the main mass of the gland. The relative size of the two lobes is very variable, not only in control animals of the same sex and litter but even in the right and left glands of the same individual. In making the nuclear counts, the smaller lobe or gland was ignored. Microscopically in the larger portion the lobules stand out more distinctly, presumably because they are separated from each other by broader trabeculae of connective tissue. The alveoli of the larger lobe are very small and belong to the so-called serous type. In the smaller lobe the alveoli are on the average larger and belong to the so-called mucous type.

The Submaxillary Gland in the Parathyroidectomized Rat

The description of Ranvier and of Loewenthal for the submaxillary of the normal rat is also valid for the glands of the parathyroidectomized and of the thyro-parathyroidectomized animals. No

qualitative differences could be noted in the histology of glands coming from controls and those from animals on which these operations had been performed. Sections cut in various planes and stained with Mallory's phosphotungstic acid hematoxylin, showed no recognizable differences in the quantity or distribution of white fibrous tissue. Likewise sections stained specifically for mucin (thionin, toluidin blue and muci-carmine) did nothing but confirm the observation of Ranvier that the larger lobe was devoid of mucous cells while the parenchyma of the smaller lobe was composed exclusively of the latter. No mitoses were seen. There was no lymphocytic infiltration or other sign of inflammation.

As just stated, simple inspection of stained sections made it seem unlikely that the enlarged size of the gland could be due to an increase in its connective tissue framework. This observation directed attention to the questions whether the parenchyma cells had increased in number or whether the individual cell had increased in size. The results reported below were obtained from glands (both experimental and control) which had so far as possible been subjected to the same histological technique. They were fixed in Zenker's fluid; an experimental and a control gland in the same bottle of the fixative and carried through all the subsequent steps together, cut the same thickness and mounted on the same slide. The animals were killed at the same time and after the same postprandial interval.

Attempts at measuring individual cells and of counting the number of cells in a unit area proved unsatisfactory. Resort was then had to counting the number of parenchyma nuclei in a unit area. The unit area was defined by a perforated piece of cardboard in the ocular. The areas were chosen quite haphazardly except that they were relatively avascular and free from larger strands or trabeculae of connective tissue. The same ocular, objective, and tube length were used throughout. In this way were counted the nuclei in ten fields at each of seven different levels (70 fields in all) of the submaxillary from the parathyroidectomized animal and in 70 comparable fields of the submaxillary from the corresponding control animal. The average number of nuclei per unit field for the parathyroidectomized rat was 11.07, while the average for the control was 16.98.

It is apparent that the individual cell of the submaxillary of the parathyroidectomized animal is considerably larger than that of the

control. Actually the ratio of their volumes is as 1.53 to 1 and the ratio of their linear dimensions would be as the cube root of these numbers or 1.15 + to 1. This means that if a particular dimension of the cell of the control animal measured 10μ, the similar dimension of the cell of the parathyroidectomized animal would measure a trifle over 11.5μ.

This increase in volume of the individual cell accounts at least in large part for the increased weight of the glands in the experimental animal. The weight of the gland from the control animal, from which these counts were made, was about 0.2120 gr. A gland, 1.53 larger, would weigh 0.3244 gr., while the actual weight of the gland of the parathyroidectomized animal weighed about 0.3781 gr.

The validity of the volume differences of the cells of the submaxillary gland of the parathyroidectomized animal and of its control, as specifically given above, is borne out by comparison with the nuclear counts obtained from glands of animals belonging to different series. The values obtained for the control and for the thyro-parathyroidectomized rats were not in all cases strictly comparable *inter se* because of technical differences, yet they indicate a cell appreciably smaller than the cell of the parathyroidectomized animal. The average nuclear count per unit field for 30 fields from the submaxillary of a thyro-parathyroidectomized animal was 17.93, while the corresponding count for the control was 15.56. These values are much nearer to that given above for the control (16.98) of the first series than are they to that for the parathyroidectomized of the first series (11.07). In other words, the parenchyma cell of the enlarged submaxillary of the parathyroidectomized is larger than those of the control or thyro-parathyroidectomized rats.

Summary and Conclusion

The enlargement of the submaxillary glands often observed in albino rats following parathyroidectomy (Hammett[3, 5]) is at least largely attributable to an increased volume of the individual parenchyma cell, i.e., a true hypertrophy. This was the only difference distinguishable between the submaxillaries of the parathyroidectomized animals and those of thyro-parathyroidectomized or of control animals.

Specifically, there was no histological evidence of any hyperemic,

fibrotic, inflammatory, proliferative, degenerative or infiltrative process.

A search of the literature has failed to reveal any other instance of submaxillary hypertrophy associated with a disturbance of parathyroid function.

Enlarged submaxillary (or other salivary) glands, described from the experimental or clinical point of view in connection with other endocrine disturbances (Horsley[6, 7]; Mikulicz[9]; Berthon[1]; Mohr[10]; Nagel[11]; Railliet[12]; Sprinzels[15]; Haemmerli[2]), apparently do not show the microscopic picture exhibited by the submaxillaries of parathyroidectomized rats.

Therefore, one is forced to the conclusion that the hypertrophy of the submaxillary glands which often follows parathyroidectomy in the albino rat is a condition *sui generis*. The data afforded by the present study seem to throw no light on the mechanism involved in this hypertrophy.

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HEMATOCELE OF THE WALL OF THE ATRIUM FOLLOWING CARDIAC PUNCTURE *

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In another article [1] the writer has called attention to the ease and safety with which blood samples may be obtained from the dog by cardiac puncture. The chief source of danger is from puncturing the larger branches of the coronary vessels and thereby producing death from a hemopericardium. The animals usually die within a few minutes with the pericardial sac distended with blood. Death from this cause may be avoided by entering the thorax sufficiently low, so that by pushing the needle upward it will be sure to enter the apex of the heart. Here there are no large vessels. We have taken over 900 samples in this way with a mortality of less than one per cent on the basis of the number of samples taken. Several dogs were bled as high as forty-five times. We also have repeatedly bled the sheep by this method. For this purpose a 16 gauge, 3 inch, Luer needle is best. Etherization in an anesthetizing chamber also facilitates the procedure in the case of the sheep.

In addition to death from hemopericardium we have had one case of hemorrhage into the wall of the left atrium, which may be of sufficient interest to justify a brief report. Death followed a few minutes after the sample was obtained. On removing the heart it was found that a large rounded mass in the wall of the atrium had completely blocked the atrio-ventricular orifice. On section it was seen that a dissecting hemorrhage had split the wall into equal portions so that the hematoma which had formed was surrounded everywhere by a layer of tissue of about the same thickness. The mass was ellipsoidal in shape and measured 4 cm. in length and 2.4 cm. in thickness in the midportion. The hemorrhage had evidently taken place from a punctured vessel within the wall of the atrium.

* Received for publication October 21, 1924.

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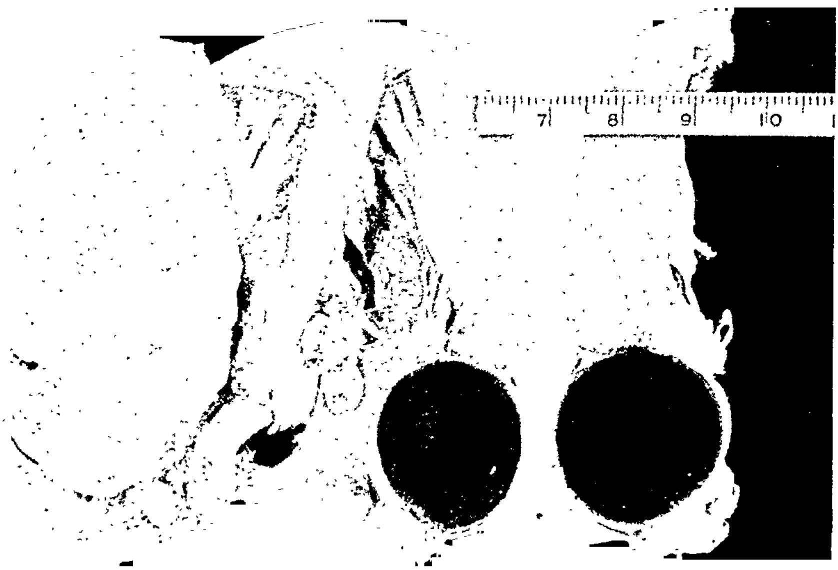
DESCRIPTION OF PLATE XLII

Figs. 1 and 2 show appearance of hematocele before and after section.



Schultz

Hematocele

THE HYDATID CYST *

THE MICRO-CHEMICAL REACTIONS OF THE HYDATID CYST WALL

Gordon Cameron, M.B., B.S., and A. S. Fitzpatrick, M.Sc.

(From the Pathological Department, University of Melbourne)

In a previous communication in 1923, one of us [1] dealt with a few of the staining reactions of hydatid cysts. Since then a more elaborate investigation into the micro-chemical reactions has been planned and the results are briefly presented here. The literature on this subject is extremely scanty, for apart from one or two short references, more or less indirect in their bearing, no work seems to have been recorded.

Echinococcus disease is fairly common in Australia, so much so that *no differential diagnosis is considered to be complete unless* due mention is made of it. The histological structure of the hydatid cyst appears, within certain limits, to be well known, and for many years the teaching of the Pathological Department of the University of Melbourne, under Professor Sir Harry Allen, has been very definite. The following description of the ordinary hydatid cyst, as found in man, may be taken as typical:

Two distinct portions are to be recognized:

1. The true cyst wall, consisting of: A. An outer layer or ectocyst, which is composed of many translucent, structureless laminae; B. An inner layer, the endocyst, or germinal layer, composed of a granular substance, presenting a cellular structure, but containing no nuclei. From this layer are derived the brood capsules, daughter and granddaughter cysts and scolices.

2. The adventitious capsule, which is simply the fibrous tissue reaction induced by the presence of the cyst, is composed of granulation tissue in all stages of development.

Daughter and granddaughter cysts arise by budding from the endocyst of the mother cyst, and have a laminated ectocyst and granular endocyst. Brood capsules have very delicate walls with no advanced specialization of layers, and may arise primarily from the mother cyst, or secondarily as buds within daughter or grand-

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daughter cysts. A scolex consists of an anterior rostellar portion with four suckers, and a double ring of hooklets, bounded behind by an hour-glass constriction, followed by a wide part which narrows rapidly into the stalk of attachment. The scolices are dotted with highly refracting particles of carbonate of lime, often double bordered or even laminated.[2] It has been the aim throughout this investigation to study the chief inorganic and organic constituents of the cyst wall by micro-chemical methods.

Methods used.

It is not proposed to describe in detail the various methods of staining. These have been dealt with, in part, elsewhere.[1] The following list presents briefly a summary of the methods employed:

Potassium	Macallum's technique.[3]
Sodium	Method of Rohdenburg and Krehbiel.[4]
Calcium	Macallum's technique.[3]
Iron:	
Inorganic iron	Prussian blue method.
Masked iron	Macallum's technique.[3]
Copper	Macallum's technique.[3]
Iodine	Justus's technique.[5]
Chlorides	Macallum's technique.[3]
Sulphates	Macallum's technique.[3]
Phosphates	Macallum's technique.[3]

Cholesterol. Fresh tissue, frozen and cut, is flooded with

formalin 30 %	2 c.c.
sulphuric acid	6 c.c.

Cholesterol gives a brownish red color in 2 to 4 minutes.

Fats: Lipoids.

Sudan III. Ciaccio's method.[6]

Osmic acid (ordinary laboratory method).

Glycogen	Langhans's method.[7]
	Best's method.[8]

The hydatid tissue was obtained from the abattoirs and from the operating theatre, so that the chief varieties were studied. In all cases the tissue was at once frozen without any fixation and then stained. A section, stained with hematoxylin and eosin, was prepared from the same tissue and employed as a check. Reagents were prepared immediately before use.

Results.

1. *Potassium.* Section showed dense black staining of the whole of the hydatid wall, i.e., a strong reaction, both of the ectocyst and endocyst. Scolices were deeply stained also.
2. *Sodium.* Sections showed extensive patchy staining of the whole of hydatid wall. Scolices were deeply stained also.
3. *Calcium.* Marked diffuse reaction in all layers and in the scolices. Reaction varied with age of cyst, and extent of degeneration. In daughter and granddaughter cysts a slight reaction was obtained; in the mother cyst the reaction was vivid.
4. *Iron.*

   Inorganic iron. Pale green staining of ectocyst, i.e., a feeble reaction. Patchy blue staining of endocyst, deep blue staining of scolices, i.e., reaction more marked than that in ectocyst.

   Masked iron. Marked reaction throughout the whole of the cyst wall, as well as in the scolices. No special arrangement of the iron in the endocyst detected.
5. *Copper.* Faint reaction in the ectocyst, more marked in the endocyst. Distribution patchy.
6. *Iodine.* Faint reaction, more marked in endocyst and scolices.
7. *Chlorides.* Faint brown staining of whole of hydatid wall affecting the spaces between the laminae of the ectocyst and all structures of the endocyst. Scolices similarly affected.
8. *Sulphates.* No staining of ectocyst, deep black staining of endocyst involving all structures. Scolices deeply stained.
9. *Phosphates.* Very faint staining of both ectocyst and endocyst and scolices, irregularly distributed. Reaction rather more marked in endocyst than in ectocyst.
10. *Cholesterol.* Faint patchy reaction in endocyst and scolices.
11. *Fats: Lipoids.* All structures gave a reaction, including scolices. This varied considerably with the age of the tissue.
12. *Glycogen.* A positive reaction to both Langhans's and Best's tests was obtained.

A brief summary is included here of the reactions to certain of the special staining methods.

(*a*) HEMATOXYLIN AND EOSIN.

Ectocyst stains with eosin.

Endocyst stains with eosin, with patchy affinity for hematoxylin.

Scolices resemble the endocyst.

(*b*) VAN GIESON.

Fuchsinophil staining of ectocyst and endocyst although some staining with picric acid in patches was also present. Scolices stain with either or both stains.

(*c*) GIEMSA.

Light purple ectocyst. Irregular blue staining of endocyst and scolices.

(*d*) GRAM'S STAIN.

Eosinophil ectocyst.

Violet and pink areas in the endocyst.

Violet scolices.

(*e*) METHYL VIOLET.

Violet ectocyst.

Light violet endocyst.

Diffuse deep violet or blue scolices.

DISCUSSION

From a micro-chemical point of view, it seems that certain conclusions can be drawn as regards the constituents of the hydatid cyst wall.

In the first place, the ectocyst differs from the endocyst in its very slight reaction to the iron, phosphate, and chlorine tests, while there appears to be no reaction to the sulphate test.

In the second place, the ectocyst resembles the endocyst in its marked reactions to the potassium, calcium, and sodium tests, and in its feeble iodine test.

The endocyst is particularly rich in potassium, sodium, and calcium. It was noticed that in the more recent daughter and granddaughter cysts, the reactions to sodium and potassium were much more vivid than those in the case of the older mother cysts. On the other hand, the reaction to calcium became more evident with increase of age of the cyst. With degeneration, calcium salts practically replace the greater portion of the cyst wall, and may be de-

tected macroscopically as small or large earthy plaques. The ectocyst shows this infiltration at a slightly earlier period and to a greater extent than the endocyst.

In order to test this observation quantitatively, analyses of the sodium, potassium, and calcium content of the cyst wall have been made. Three types of cysts have been examined:

1. Daughter and granddaughter cysts.
2. Mature mother cysts.
3. Cysts which have just commenced to degenerate.

Number 1 represents the youngest variety of cyst, number 3 the oldest. The ordinary methods of analysis as described in Mellor's "Quantitative Analysis," were used. The results are as follows expressed in terms of percentage of undried weight:

	Calcium	Alkalies	Sodium	Potassium
1	0.465	0.678	0.607	0.071
2	0.959	0.455	0.389	0.066
3	1.968	0.252	0.152	0.100

From these figures it is plainly seen that the conclusion drawn from the micro-chemical tests is correct, with the exception that the potassium content of the degenerating cyst wall was somewhat higher than that in the young cyst. The hydatid cyst thus does not materially differ in this respect from certain other new growths, especially tumors, as shown by the work of Clowes and Frisbie [9] and of Beebe.[10] But there appears to be a considerable difference in the potassium content, which is so much less in the hydatid cyst wall. It will be seen that the marked potassium, sodium, and chlorine reactions go side by side with the high content of these substances in the hydatid fluid. It seems fairly certain that they are derived from the cyst wall, being secreted along with fluid into the cyst cavity.

A reaction for iron, both inorganic and organic, was well marked in the endocyst and scolices, less evident in the ectocyst, and this was associated with a faint, patchy reaction for copper. From its distribution, and from the much more vivid Prussian blue reaction, it seems likely that iron is present mainly in the form of inorganic compounds.

Cholesterol is often abundant in the fluid and a reaction to cholesterol in the cyst wall is given.

The glycogen reaction has been reinvestigated, using in addition Best's carmine method, and a confirmatory result obtained. It seems then, that glycogen is distributed throughout the whole of the cyst wall, in much the same way as fat.

Definite positive results were obtained, both with the Sudan III and the osmic acid methods, so that fats are present in the cyst wall and scolices. These reactions also become much more marked as the cyst grows older, and with degeneration there is extensive fatty change throughout the whole of the cyst wall, preceding the calcareous infiltration from the periphery of the ectocyst.

It has already been pointed out that an extensive fat reaction is sometimes found in the liver when a hydatid cyst is present.[1] This has been investigated in a number of cysts, and the conclusion reached that such a degeneration is by no means uncommon. The intensity of the fatty change depends to a large extent on the period over which the liver has been infected by the cyst. Apparently toxic substances are liberated during the growth of the cyst, giving rise to the leucocytic reaction as well as the degenerative changes, if continued over a long time.

With all methods of investigation, no indication of a nucleus could be obtained.

In regard to the special staining reactions, reference might be made to the affinity of the ectocyst for eosin. This is seen in the case of all composite staining methods in which eosin is made use of, e.g., Giemsa's method.

With all methods of staining, the scolices resemble closely the endocyst, a result which might be expected from the fact that the former are constantly developing from the latter.

The nature of the hydatid fluid will be the subject of a future paper.

Conclusions

1. The ectocyst of the hydatid cyst wall is (from micro-chemical observation) rich in potassium, sodium, and calcium, poor in iron, iodine, phosphates, and chlorides.
2. The endocyst shows well-marked reactions to tests for each of the above.
3. As the hydatid cyst grows older, the sodium and potassium

salts become less in quantity, while calcium salts, fats, neutral and unsaturated, and lipoids increase.

4. Iron is present mainly in the form of inorganic compounds.

5. Glycogen is distributed throughout the whole of the cyst wall, in much the same manner as fat.

6. Scolices closely resemble the endocyst in their micro-chemistry.

Acknowledgments

We are indebted to Professor Sir Harry Allen and Acting-Professor F. L. Apperly for their interest and many suggestions.

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THE ORIGIN OF ADENOMATOUS GOITRE *

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There are two principal views concerning the origin of adenomatous goitre (struma nodosa). In the opinion of Beck,[1] Wölfler,[2] Ribbert,[3] etc., this condition arises from multiple embryonal cell nests scattered through the thyroid tissue, especially toward the periphery, which begin to enlarge from the time of puberty onward. According to the hypothesis of Virchow,[4] Hitzig,[5] Michaud,[6] etc., the development of the adenomatous nodules is from the adult thyroid tissue. From a microscopic study of over six hundred adenomatous goitres at Lakeside Hospital, the impression is gained that the latter view is correct.

Virchow in 1863 described the process as follows: "The cells of the acini increase by division in various portions of the acini. In this way solid plugs arise which push themselves outward, penetrate the soft interstitial tissue, again form new plugs and branch more and more. The interstitial tissue can be involved in the stimulation, proliferate and cut off portions of the plugs. . . . Later the plugs become hollowed out, fluid is secreted and they become active as acini. . . . If the development is uniform throughout the gland, the outer surface will appear comparatively smooth. . . . If it is unequal, a lobulated, nodular, knobby condition will result. The size of the nodules varies extraordinarily, according to the number of involved lobules, according to the degree of proliferation, and according to the secondary enlargement of the separate outgrowths and acini. Many nodules are barely pea-sized, others reach the size of a man's fist. If they lie at the periphery, they gradually push themselves away from the rest of the gland and at times come to lie quite independent of it. If they have a more central location, they squeeze the neighboring lobules together, compress them, so that these frequently form concentric zones about the nodules and finally lead to their atrophy. . . . In my opinion there are two en-

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tirely different orders of development: The capsular connective tissue formation arising from the old interstitial tissue and the landular hyperplasia arising from the old parenchyma."

According to Hitzig, "(1) The beginning of struma formation really depends upon proliferative processes in the normal colloid-containing and colloid-free glandular epithelium. (Virchow as against Wölfler): (2) The first definite appearances of struma nodosa are individual tubes of specifically differentiated epithelium which occupy the place of the normal tissue of the secondary lobules. (3) Furthermore, this tissue, more and more metaplastic, replaces the normal tissue of a secondary or even a primary lobule. (4) The lobules so altered, increase in volume, assume a spherical shape and displace the neighboring tissue. These masses constitute the smallest real struma nodules. (5) In the neighborhood, in a similar manner, altered lobules can form multilocular composite struma nodules by fusion (*a*) by the growth of the tubes through the septa, (*b*) by compression of lobules leaving the outer surface round, or finally (*c*) by growth and enlargement of compressed lobules surrounding central larger ones. (6) The metaplastic formation of the goitre tissue ends in the nodule when the boundaries of the primarily involved lobule are reached; then a growth of the nodule takes place from within itself with displacement of the surrounding tissue. (7) The diffuse goitre depends upon a similar proliferation in all lobules of the gland. (8) The nodular struma is brought about by a difference in growth energy of the different adjoining portions. (9) The greatest variety of transitions between the two kinds occurs."

In a microscopic study of over six hundred adenomatous goitres at Lakeside Hospital, adult thyroid tissue was observed apparently passing from its normal lobulation by gradual changes to circumscribed, encapsulated nodules histologically indistinguishable from adenomata.

In sections of thyroid glands showing varying degrees of hypertrophy and hyperplasia, more or less circumscribed areas are not infrequently found in which the hyperplasia is more marked than generally throughout the gland. These circumscribed areas may comprise one or more lobules or portions of lobules. In addition the repeated change in glands from a hyperplastic and hypertrophic condition to the colloid state apparently brings about in some an increase in stroma around and within lobules causing greater cir-

cumscription of lobules than normal, or division of lobules into smaller circumscribed units. It is possible that the changes just mentioned may underlie the initiation of adenomatous goitre, for, when hyperplasia occurs in these circumscribed areas (most marked usually at the periphery) they gradually increase in size and become spherical in form.

For a while the only encapsulation may be by the normal regional stroma. A definite capsule is gradually acquired by one of two processes apparently: (1) The compression of regional thyroid tissue with atrophy and replacement fibrosis thereof. (2) When the growing lobule returns to the colloid state, the older central acini usually take up much more colloid than the recently formed peripheral ones. The pressure exerted toward the periphery thereby, results in atrophy and replacement fibrosis of the outer acini with consequent increase in thickness of the perilobular stroma.

In adenomatous goitres there are many circumscribed, encapsulated nodules of this sort in which the relation of the acini to one another is similar to that in the normal thyroid gland. These nodules show the earliest transformation from normal thyroid tissue. It has not been possible, however, to decide whether a metaplasia occurs in the sense of Hitzig or whether this early change depends, as Virchow thought, merely upon the ordinary process of growth. In addition, in the ordinary adenomatous goitre, further stages of tumor formation are noted in which the central portions of the spherical lobules show congestion of the vessels, edema of the stroma, and separation of the acini from one another and from blood vessels. This congestion and edema of the lobule with disturbance in relationship of acini to one another and to blood vessels is a characteristic one and sharply marks off the adenomatous lobules from those of the normal thyroid gland. In addition to nodules of this type there are others varying in diameter up to 2 to 3 cm., showing gradations from these to tumors in which the congestion, edema, and separation of acini from one another and blood vessels are present from the center to the capsule, which at this stage is usually greatly thickened, sometimes to more than a millimeter. In this stage the histology of the nodules is indistinguishable from that of large single differentiated fetal adenomata. Furthermore, the same degenerative changes, such as central parenchymatous atrophy with great increase in edematous stroma with subsequent hyalinization

and even calcification of this tissue, are frequently present; hemorrhage and cyst formation also occur.

Conclusion

From a microscopic study of over six hundred adenomatous goitres, there is no evidence that this condition depends as contended by Beck, Wölfler, Ribbert, etc., upon a development after puberty of embryonic thyroid cell nests. On the other hand, the impression is gained from the study that adenomatous goitre arises, as suggested by Virchow, Hitzig, Michaud, etc., from adult thyroid tissue. Furthermore, there is evidence for their belief that it depends essentially upon an irregularity in the degree of proliferation of acini in different portions of the gland.

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DESCRIPTION OF PLATES XLIII and XLIV

1. Gross photograph of adenomatous goitre.

2–10. Photomicrographs illustrating possible origin of adenomatous goitre.

2. Lobules divided into smaller units, stroma increased, in return of thyroid from hyperplastic to colloid state.
3. Irregularity in degree of proliferation of acini in different portions of a lobule.
4. Early adenomatous transformation of lobule or portion of lobule (stage of hyperplasia). Lobule assuming spherical form. More marked hyperplasia at periphery. Normal relationship of acini to each other and to vessels.
5. Early adenomatous transformation of lobule or portion of lobule (colloid state). Older central acini contain more colloid than more recently formed peripheral ones. Normal relationship of acini to each other and to vessels.
6. Moderately advanced adenomatous transformation with separation of central acini from each other and from vessels.
7. Separation of acini from each other and from vessels due to congestion and edema.

8. Considerably advanced adenomatous transformation with separation of acini from each other and from vessels, reaching almost to capsule. Capsule well defined.
9. Advanced adenomatous transformation with separation of acini from each other and from vessels, reaching to capsule. Capsule thick.
10. (Formation of capsule.) Older central acini distended with colloid. Atrophy and replacement fibrosis of more recently formed peripheral acini.
11. Microphotograph of large single partially differentiated fetal adenoma (true tumor) with separation of acini from each other and from vessels due to congestion and edema.
12. Fetal adenoma (same as 11) with separation of acini from each other and from vessels, reaching to capsule. Capsule thick.

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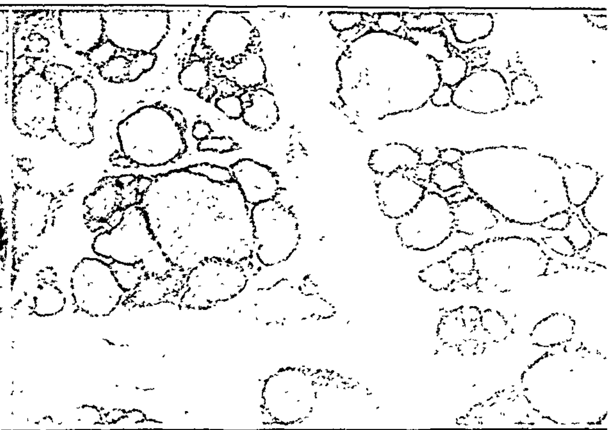

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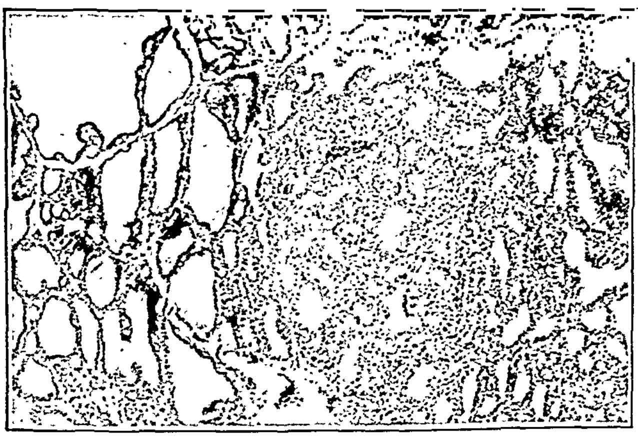

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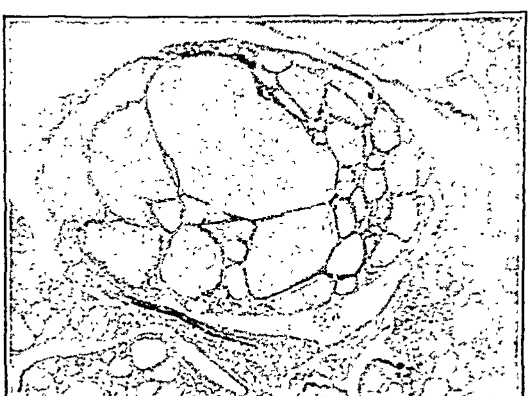

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Kline

Adenomatous goitre

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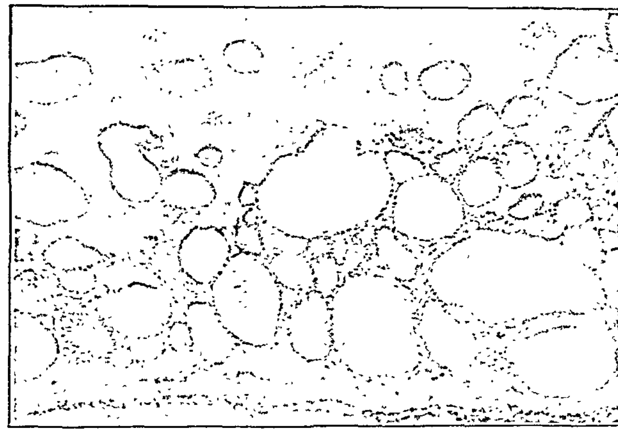

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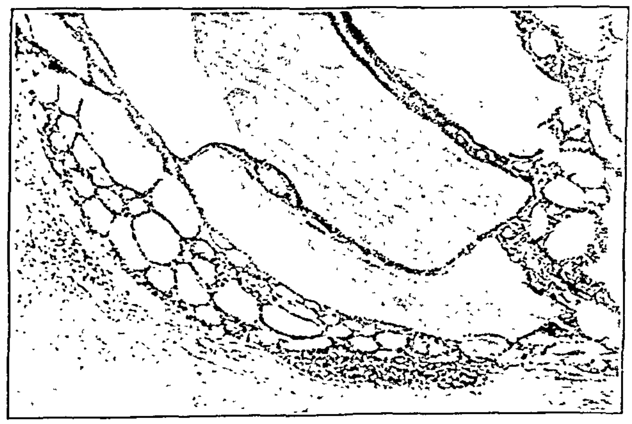

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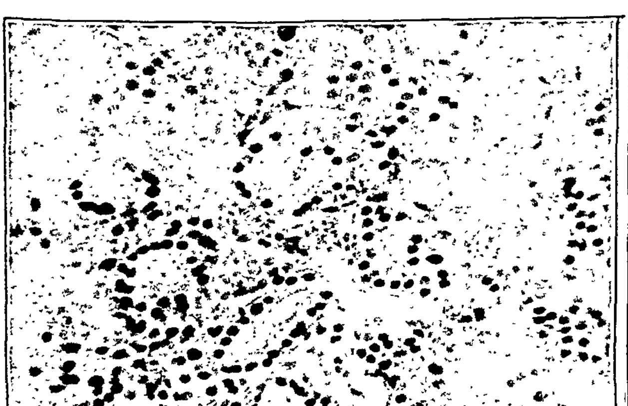

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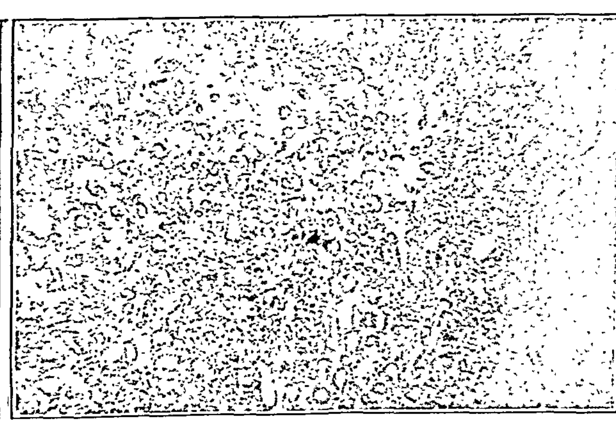

12

Kline

Adenomatous goitre

THE ETIOLOGY OF ACUTE APPENDICITIS *

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In spite of the frequent occurrence of appendicitis and the thorough clinical and pathological understanding of the disease, there is still considerable discussion as to its etiology. Aschoff [1] believes the streptococcus, either alone or associated with a slender, curved Gram positive bacillus, to be the causal agent. McWilliams[2] took swabs from the appendicial serosa at the time of operation and cultured the swabs. Of 288, 57 per cent yielded B. coli, 19 per cent B. coli and Streptococcus, and 8.3 per cent a streptococcus alone. Kelley [3] found B. coli in the majority of pathological appendices he cultured.

Most of the work done on this subject has been purely bacteriological or purely histological. The advantage of combining these two methods of study is obvious, and the procedures described below were adopted as best suited to demonstrate the presence of organisms and to provide an accurate check against one another.

Methods. The excised appendix was dropped by the surgeon into a sterile Petri dish and then carried to the laboratory, care being taken to avoid contamination with material from the lumen. Occasionally when appendices were removed at night a few hours elapsed before they were examined. However, these were kept cold, and controls showed very little change to occur.

The serosa, and exudate covering it, if any, was dissected off with sterile precautions, over an area about 1 cm. square. That portion of muscularis thus exposed was removed, taking so far as possible the entire thickness of the muscle without breaking through into the mucosa. Finally a bit of mucosa together with some of the contents of the lumen were removed.

Each of these pieces of tissue was smeared over the surface of a blood agar plate, and then divided, one portion being dropped into a tube of peptone broth and the other into a tube of previously heated and cooled peptone broth which was overlaid with sterile

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petrolatum after inoculation. The organisms which developed under these various conditions were studied and classified.

The appendix was fixed *in toto* in Zenker's fluid and paraffin sections made from various portions, always including the immediate vicinity of the tissue taken for culture. Duplicate sets of sections were stained with eosin-methylene blue and by the Gram-Weigert method.

The cultural findings were then checked against the histological. No obvious discrepancies were encountered, though naturally organisms were sometimes not found in the sections when cultures showed them to be present.

Material. A large number of appendices removed by members of the Surgical Staff of the Boston City Hospital from cases diagnosed as acute appendicitis were examined and one hundred selected. Symptoms had existed in these cases from a few hours to a few days, from 12 to 48 hours in the majority. Most appendices showing little or no evidence of inflammation were discarded, though a few were included in the series for control purposes.

Of the 100 appendices, 66 were diagnosed histologically as acute appendicitis, 4 as acute periappendicitis, 15 as healing appendicitis, 1 as healed appendicitis with obliteration of the lumen with recurrent healing appendicitis, 1 as chronic periappendicitis (tuberculous), 10 as healed appendicitis, of which one showed healed fibrous tubercles, 2 as healed appendicitis with obliteration of the lumen, and 1 as healed appendicitis with acute inflammation of a nearby mesenteric lymph node.

None showed evidence of parasitic worms in the lumen. From casual observations in this laboratory, we have the impression that parasitic worms very rarely are found in acute appendicitis, though present in about 2 per cent of negative or healed appendices.

We regard the following as the minimum requirements for a diagnosis of the various conditions:

- for acute appendicitis, pus in the lumen and some evidence of inflammation in the wall;
- for healing appendicitis, fairly numerous eosinophiles in the muscularis, also foci of lymphocytes;
- for healed appendicitis, focal collections of lymphocytes in the muscularis or serosa, either inside or outside lymphatics.

The diagnosis chronic appendicitis is not made. The dividing line between acute and healing appendicitis is not very clear-cut, but the lesion is called healing when there is a predominance of eosinophiles over polymorphonuclear leucocytes in the exudate and evidence of proliferation of fibroblasts. The presence of eosinophiles in the mucosa is considered normal.

Observations. The bacteriological findings in the 66 acutely inflamed appendices show B. coli and Streptococcus hemolyticus, with or without B. coli, to be most frequently encountered. In Table 1 are presented the bacteriological results. In two instances organisms

TABLE 1

Organisms present in the wall in acute appendicitis. 66 cases

	Serosa	Muscularis	Mucosa	Total
B. coli communis	14	17	18	18
B. coli communior	7	7	7	7
Streptococcus hemolyticus	5	5	5	6
Streptococcus hemolyticus + B. coli	9	9	9	9
Proteus vulgaris	4	4	5	5
B. pyocyaneus	4	4	4	4
B. pyocyaneus + B. coli	1	1	1	1
Streptococcus viridans	3	2	3	3
Streptococcus viridans + B. coli	1	1	1	1
Staphylococcus albus + B. coli	1	1	1	1
Pneumococcus type I	1	1	1	1
Streptococcus (non-hemolytic)	0	0	0	1
No growth	..	..	..	9

were found in the lumen only — Streptococcus hemolyticus in one case and a non-hemolytic streptococcus in the other. No typhoid appendices were encountered, in spite of fairly numerous cases of typhoid fever admitted to the hospital.

The four cases of acute periappendicitis all occurred in females. From the serosa of two of the appendices Pneumococcus type I was recovered. One of these two cases, a girl of five years old, developed a lobar pneumonia four days after operation and Pneumococcus type I was recovered from the sputum. Another of the cases of acute periappendicitis yielded Streptococcus viridans, and in the last (who gave a history of pelvic inflammation) the gonococcus was found to be the causal agent.

Of the sixteen cases of healing appendicitis, one showed B. coli and one Streptococcus viridans, both present in the serosa and not in

the muscularis. These two appendices were not far removed from the acute stage of the process. The remainder showed no growth. Organisms were found in only one of the appendices in the healed stage occurring throughout the wall, and that one was left twelve hours at room temperature to determine how far organisms in the lumen would grow out into the wall.

A lymph node accompanying one appendix, which was diagnosed as healed, showed evidence of acute inflammation, and B. coli was recovered from the node.

Discussion. The earliest stage of the lesion found in these acutely inflamed appendices is necrosis and slight ulceration of the mucosa, not in those portions nearest the center of the lumen, as would be expected if a bacterial poison present in the lumen were causing the change, but down in the folds, somewhat suggesting the early inflammatory changes in the crypts of the tonsil. All of the organisms found in the lesions, with the exception of a pneumococcus, are fairly common in the intestinal flora. There is little need, therefore, to hypothecate a hematogenous origin of the infection. The character and location of the early lesions are decidedly in favor of an invasion from the lumen.

The peristaltic action of the muscularis of the appendix is probably of great importance in keeping the contents of the lumen in motion, and any interference with this action, whether functional or structural, due perhaps to scarring from previous attacks, will favor the accumulation of bacteria in large numbers and ultimately permit sufficient concentration of bacterial products to cause necrosis of adjacent epithelium. Obstruction of the lumen, whether by kinking, partial obliteration by scarring, or the presence of fecoliths, would have the same effect.

The occasional appendices which showed no organisms either on culture or histologic examination and yet were acutely inflamed can probably be explained by the destruction of the invading organisms by the defense mechanism of the body, but only after they had done serious damage.

One striking feature is the similarity of the exudate in all acutely inflamed appendices, in spite of the various organisms calling it out. In this small series, at least, it is impossible to pick out any one organism as tending to produce a given type of appendicitis. The

factors giving rise to a phlegmonous inflammation as against an ulcerative, for example, are not etiological but must be sought for in anatomical peculiarities or in the locations of the invading organisms. The mucosa is apparently much more resistant than the muscularis or serosa, and the infection often spreads in these layers outside the mucosa, so it is not unusual to find a lesion that appears phlegmonous in one section to be ulcerative in another. I have the impression, however, that the exudate is decidedly more hemorrhagic and necrosis more widespread in those appendices in which Streptococcus hemolyticus was found, but no definite line can be drawn. Such findings are, however, entirely in keeping with streptococcus lesions elsewhere.

The same similarity holds for the healing process. The predominance of eosinophiles in the exudate, once repair begins, appears to be a peculiarity of the appendix, as they are rarely encountered in any numbers in other portions of the intestinal tract. What the reason for their constant occurrence in healing appendicitis may be I have been unable to determine.

As mentioned above, the time between onset of symptoms and removal of the appendix varied considerably in these cases. As a rule there was a fairly close correspondence between the duration of symptoms and the pathological findings. The cases operated on within a few hours of the onset usually showed only a small area of necrosis and ulceration with leucocytic infiltration. From then up to forty-eight or seventy-two hours there is no evidence of repair other than slight proliferation of fibroblasts, which is rather a response to injury than a definite attempt at repair. Those appendices removed four to eight days after onset of symptoms generally showed active fibroblastic proliferation, a predominance of eosinophiles in the exudate, and mitoses in the glands, if any epithelium were left. A few showed no evidence of healing up to the sixth day, and one was completely gangrenous.

Those cases diagnosed pathologically as healed appendicitis usually gave a history of one or more previous attacks dating back weeks to months, and sudden pain of a few hours duration immediately preceding operation.

Though the cases are not included in this series, we have recently had evidence that acute appendicitis can exist without noteworthy symptoms. One appendix removed routinely with chocolate cysts

of the ovary and vaginal wall was found to have pus in the lumen with necrosis, ulceration and leucocytic infiltration of the mucosa at several points and slight infiltration of the muscularis. Another removed with a fibroid uterus was even more inflamed. Neither of these cases had given the surgeon reason to suspect any acute abdominal condition.

Summary and Conclusions

1. The bacteriological and histological results from a study of 66 cases of acute appendicitis show B. coli to be the organism most commonly encountered, being found alone in 25 appendices and combined with other organisms in 12. Streptococcus hemolyticus occurred alone in 6 and combined with colon in 9. Proteus vulgaris and B. pyocyaneus were each found five times. There was a scattering of other organisms.
2. All early lesions found were at the margin of the lumen.
3. The inflammatory reaction does not vary with the organism found in the lesion, although it is perhaps more hemorrhagic in those cases where Streptococcus hemolyticus was recovered.
4. In this series of cases the delicate Gram-positive bacillus described by Aschoff was not found in the lesion and only rarely in the contents of the lumen.
5. One case of Pneumococcus type I periappendicitis in a five-year-old girl was followed in four days by lobar pneumonia, also due to Pneumococcus type I.
6. The evidence is against a hematogenous origin of acute appendicitis.
7. Acute appendicitis is not a specific disease due to one type of organism, a streptococcus as sometimes maintained. Like acute tonsillitis, acute endocarditis and similar infectious lesions, it may be caused by a variety of organisms.
8. Acute appendicitis may occur without symptoms.

I am indebted to Dr. R. C. McLeod of this laboratory for help in preparing and examining some of the specimens, and to the Surgical Services of the Boston City Hospital for the material used.

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EXPERIMENTAL GLOMERULONEPHRITIS *

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There are four anatomic types of renal disease in man that are commonly called nephritis. These differ in etiology as well as in clinical and anatomic features, and it is therefore important to distinguish them sharply in an experimental study.

1. Exudative interstitial nephritis (pyelonephritis, abscess). This is an ordinary exudative inflammation of the kidney in which bacteria are readily demonstrable. Acute interstitial nephritis is a special type of exudative inflammation most frequently observed following scarlet fever.

The exudate, composed of polymorphonuclear and mononuclear leucocytes, compresses and destroys portions of the tubules and may in this way produce areas of atrophy in chronic cases. In man contracted kidneys are rarely if ever produced by this disease; but in the laboratory animals this is the only known form of contracted kidney.

The spontaneous nephritis [1] of the rabbit is of the exudative interstitial type. It is readily produced by intravenous injections of streptococci and seems to develop frequently when the kidneys are injured by toxic chemical substances such as uranium nitrate. This is the only form of contracted kidney that has been obtained by experimental procedures. It is a chronic nephritis but it is quite different histologically from the human contracted kidney.

2. Nephrosis. This is a degenerative change, affecting chiefly the tubules, but to some extent the glomeruli also, and varying in intensity from cloudy swelling to widespread necrosis. There is no

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inflammatory reaction. It is usually secondary to some other disease, and the kidney commonly recovers readily when the primary condition heals. A contracted kidney never develops. The injurious substances are bacterial toxins, chemical poisons (uranium, mercury, etc.), and metabolic poisons (as in diabetes and the toxemia of pregnancy). A nephrosis is easily produced experimentally by bacterial or chemical poisons. Experimental uranium nitrate nephritis belongs to this group.

3. The arteriolosclerotic kidney (hypertensive kidney, primary contracted kidney). About three fourths of the cases of primary hypertension show this renal lesion to some extent, but it is seldom far enough advanced to produce any measurable disturbance of renal function. A true primary contracted kidney is rare. The structural change consists in sclerosis of the afferent glomerular arterioles and small arteries with resulting atrophy of glomeruli. After the glomerulus has ceased to function its tubule atrophies and when the atrophy is extensive a contracted kidney results. Nothing definite is known about the etiology of this disease. It has not been produced experimentally.

4. Glomerulonephritis [2] is an inflammation of the kidney, chiefly restricted to the glomeruli. The principal change is a swelling and proliferation of the endothelium resulting in closure of the glomerular capillaries, but there is often a marked proliferation of the cells of the outer layer of Bowman's capsule, giving rise to epithelial crescents, and there may be a notable accumulation of polymorphonuclear leucocytes in the tuft and capsular space. The inflammatory reaction may therefore be described as proliferative and exudative. The closure of all the capillaries of a glomerulus is soon followed by disuse atrophy of its tubule, and it is this tubular atrophy which is largely responsible for the striking gross and microscopic appearances in chronic glomerulonephritis. The microscopic picture is very characteristic and with rare exceptions it is readily distinguished from any other form of renal disease.

Glomerulonephritis includes all the acute and subacute and most of the chronic cases of what is commonly called Bright's disease. Some cases of chronic Bright's disease belong in the group of arteriolosclerotic kidneys. Contracted kidneys in man are usually instances of chronic glomerulonephritis, but a few are of the arteriolosclerotic type.

Glomerulonephritis apparently does not occur in the laboratory animals, but it has been found in cattle and horses. [3] We have not been able to find any literature dealing with nephritis in monkeys.

An excellent review of the literature dealing with experimental nephritis has recently been given by Leiter, [4] to whose paper the reader is referred. It is evident that the only forms of nephritis that have been produced experimentally are exudative interstitial nephritis (corresponding to human pyelonephritis) and nephrosis. A true glomerulonephritis has not been obtained experimentally. The nearest approach to success was by Pernice and Scagliosi [5] who produced a few epithelial crescents in a dog by subcutaneous injections of B. pyocyaneus. Baehr [6] obtained epithelial crescents also by subcutaneous injections of uranium. But in both these instances only a few glomeruli were involved. There was no widespread glomerular inflammation, and the lesions were evidently early, since no glomerular hyalinization or tubular atrophy is mentioned.

Material and experimental procedure. Twenty-five rabbits and fourteen young rhesus monkeys were used. Streptococci from human lesions were grown on blood agar slants 24 to 48 hours, and suspensions of the growth were then made in physiologic salt solution. Nonhemolytic streptococci were generally used, since they seemed somewhat less virulent. The suspensions were made fairly uniform in density so that the dosage could be estimated roughly by the number of cubic centimeters injected. A heavy dose for monkeys was from 10 to 15 c.c. of a dense suspension of organisms washed from blood agar slants. The number of slants used varied with the heaviness of the growth, from 3 to 10 slants for a heavy inoculation. Injections were given intravenously and were repeated every few days until a moderate albuminuria developed. The inoculations were discontinued temporarily when the animal showed evidence of toxemia or when a heavy albuminuria was present.

Ten strains of streptococci were used in these experiments. Three of these were hemolytic and seven were nonhemolytic (Streptococcus viridans).

Hemolytic strains: Organism 342 was isolated post-mortem from the blood of a patient dead of septicemia following erysipelas. Organism 4 was obtained at post-mortem from the pericardial exudate of a patient dead of acute rheumatic fever and acute pericar-

ditis. Organism 29 was isolated from the throat of a patient with scarlet fever. The toxin of Organism 29, diluted 1–800, gives a strongly positive Dick test in 0.2 c.c. doses.

Nonhemolytic strains (Streptococcus viridans): Organism 2 was isolated from the blood of a patient with chorea. Organism 1 was obtained from the blood of a patient with acute rheumatic endocarditis. Organism 8 was isolated from the blood of a patient with acute rheumatic fever and endocarditis; and Organism 9 was obtained from the blood of a similar case which also had a pericarditis. Organism 5 was isolated from the exudate of a joint in a case of acute rheumatic fever. Organism 18 was obtained post-mortem from the blood of a patient dead of acute rheumatic fever with acute rheumatic endocarditis; and Organism 11 was obtained post-mortem from the blood and pericardial exudate of a similar case in which a pericarditis also was found.

Experiments with rabbits. It seems unnecessary to give these experiments in detail since the results were negative. About 25 rabbits were used and a large number of injections were given some of them. Several animals died of endocarditis. Albuminuria was readily produced. Renal lesions were frequently found at post-mortem, but they were always of the ordinary spontaneous type, that is, lymphocytic interstitial nephritis.

Experiments with monkeys. No. 10. Young female. May 21, 1924. Heavy inoculation with Organism 18. Death about 12 hours later from bacteriemia. Kidneys normal.

No. 5. Young female. April 23, 1924. Heavy inoculation with Organism 11. Death about 12 hours later from bacteriemia. Kidneys normal.

No. 6. Young female. April 23, 1924. Urea nitrogen 22.40 mg.; creatinin 1.80 mg. Urine: no albumin. Injected with Organism 8, April 24 and 25. April 26, urine −, albumin +; very sick. April 29, death from bacteriemia. Kidneys greatly swollen and opaque. Microscopic: extensive tubular necrosis, occasional necrotic areas in the glomeruli. No glomerulitis. Diagnosis: severe nephrosis.

No. 4. Young female. March 25, 1924, injected with Organism 11. March 27, second injection. March 28 to April 1, severely ill. April 1, death from bacteriemia. Kidneys slightly cloudy and swollen. No glomerulitis.

No. 12. Small female. June 4, 1924, no albumin. June 10, injected with Organism 29, small dose. June 12, severely ill; albumin +; infection of right eye. June 17, albumin ++; marked swelling and reddening of both lower extremities, especially around the ankles; purulent infection of right eye. June 18, lies prostrate on floor of cage; enormous swelling of ankles. June 21, urea nitrogen 59.3 mg.; creatinin 3.0 mg. Death, June 21. Large quantities of pus in and around ankle joints. Some pus around tendons of right wrist. Swollen kidneys with very opaque cortices. Microscopic: extensive infiltration of the cortex with medium-sized lymphocytes and plasma cells. The exudate is not uniformly distributed but is in irregular areas, spreading out from the small blood vessels. The largest accumulations of lymphoid cells are perivascular but there are large numbers between the tubules and around the glomeruli in the cortex (Fig. 1).

The microscopic appearance corresponds to the acute interstitial nephritis of scarlet fever. It differs from the spontaneous nephritis of the rabbit chiefly in its wide distribution through the cortex. Diagnosis: acute interstitial nephritis.

No. 11. Female. June 4, 1924, no albumin; urea nitrogen 14.0 mg.; creatinin 2.1 mg. Injected with Organism 18. June 2, second injection; violent reaction a few hours later. June 9, no albumin; third injection. June 11, no albumin. June 16, no albumin; fourth injection. June 18, albumin +; fifth injection; violent reaction about two hours later. June 19, did not recover from last injection. Death. Kidneys normal.

No. 13. Female. June 21, 1924, no albumin. Small injection of Organism 29. June 24, no albumin; second injection. June 26, third injection. June 27, no albumin. June 30, no albumin; fourth injection. July 2, trace of albumin. Death, July 4. Post-mortem diagnosis: generalized tuberculosis. Kidneys normal.

No. 7. Young male. April 26, 1924, no albumin. April 28, urea nitrogen 12.13 mg.; creatinin 2.14 mg.; injected with Organism 11. April 30, albumin +. Injections were given May 4, 8, 10, 12, and 15. May 11, no albumin. Suppuration developed in one leg at the site of inoculation. Blood chemistry, May 15: urea nitrogen 40. mg.; creatinin 1.8 mg. Death May 15, from bacteriemia. Swollen kidneys. No glomerulitis. Diagnosis: mild nephrosis.

No. 8. Female. May 21, 1924, no albumin; urea nitrogen

22.4 mg. Injected with increasing doses of Organism 9 on May 21, 22, 26, 27, 28, 29, 31, June 2, 4, 6, 9, 11, and 16. June 4, no albumin. Death June 16. Kidneys normal.

No. 14. Female. June 21, 1924, no albumin. Small injection of Organism 29. June 24, no albumin; small injection. June 26, small injection. June 27, trace of albumin. June 30, albumin +. Moderate-sized injections July 9, 11, 14, 15, 17, 22, 26, August 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 18, and 19. Urine: no albumin July 11, 14, 17, 26, August 4, 12, and 15. August 19, albumin ++. During the last few days evidences of toxemia developed and death occurred at 7 P.M. August 19. Kidneys a little cloudy. Microscopic: occasional necrotic tubules, a few casts. No glomerulitis. Diagnosis: nephrosis of moderate degree.

No. 9. Male. May 21, 1924, no albumin; urea nitrogen 19.6 mg.; injected with Organism 342. May 22, second injection; joints of both lower and the right upper extremities swollen and tender. Injections May 26, 28, 31, and June 2. Albumin +, May 26. No albumin June 2; joints still swollen. Injections June 4, 6, 9, 11, 16, 17, 23, 24, 26, 27, July 2, 3, 9, 11, 14, 15, 17, 18, 22, 26, August 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 19. No albumin June 17, 23, 26, July 2, 11, 14, 17, 26, August 4, 12, 14, and 16. August 19, albumin ++. Stuporous after August 18. Death August 20. Kidneys about twice normal dimensions; soft hemorrhagic cortices. Microscopic: necrosis of the greater part of the cortices. Blood in all the tubules. An occasional glomerulus shows acute glomerulitis. Diagnosis: severe nephrosis (necrotic type) with very restricted acute glomerulonephritis.

No. 1. Female. Injections with Organism 2, 1st, 4th, 7th, 10th, and 13th days of experiment. No noticeable clinical effect; 13th day, no albuminuria. Injections with Organism 8, 20th, 21st, 24th, and 28th days. No clinical effects; no albuminuria. Injections with Organism 4, 31st, 33d, 35th, 36th, 38th, and 41st days. The animal became very sick after the first injection of Organism 4, and showed some reaction at each subsequent injection. No albuminuria. Injections with Organism 11, 54th, 55th, 66th, 69th, 71st, 73d, 76th, 80th, 89th, 90th, and 92d days. Urine, albumin: 83d day +; 88th +; 92d +; 94th +. Death, 95th day. Blood chemistry, 95th day: urea nitrogen 97.0 mg.; creatinin 2.7 mg.

There were no striking clinical effects from the injections until

Organism 11 was used. After each inoculation with this organism there was a very severe reaction; the animal would vomit and lie on the floor of the cage for several hours. There was a rapid loss of weight. During the last week suppuration developed in one leg at the site of inoculation.

At post-mortem 100 c.c. of clear fluid was found in the peritoneal cavity, but there was no fluid in the other serous cavities and no edema. The liver and the heart showed a faint cloudy swelling. The kidneys were not enlarged but they were very pale and cloudy. Microscopically there was no necrosis of the tubules or glomeruli and no exudate. The glomerular endothelium was swollen, but there was no definite glomerulitis of any kind.

No. 2. Female. Injections with Organism 5. March 1, no albumin. Injections were given March 1, 3, 5, 7, 10, 13, 17, 19, 21, 22, 26, 27, 30, and 31. April 1, no albumin. Injections April 2, 4, 5, 7, 10, 23, 24, 26, 28, and 30. May 1, trace of albumin; marked loss of weight. There was a slight reaction after each injection during March and April but there were no violent reactions.

Injections May 3, 5, 8, 10, 12, 15, 19. Urine, May 7: albumin++; May 11, albumin +++; May 19, albumin +++; May 22, albumin +++. Blood chemistry May 19: urea nitrogen 60.7 mg.; creatinin 2.1 mg. During May the reaction following each injection was more severe and emaciation became extreme.

Very small injections were given June 2 and 4. These were followed by severe reactions. Urine: June 2, albumin +++; June 6, albumin +++. Blood chemistry June 11: urea nitrogen 36.4 mg.; creatinin 2.5 mg.; June 16, urea nitrogen 49.3 mg.; creatinin 3.0 mg. Death June 16.

At post-mortem there is marked general emaciation. There is no edema and no fluid in the serous cavities. The heart and liver are faintly cloudy. The heart is of normal size.

The kidneys are moderately enlarged and their capsules are slightly adherent. The surfaces are finely granular. The cortices are opaque in appearance and no markings are visible. On microscopic examination most of the tubules show a definite reduction in size, but those connected with badly damaged glomeruli show a pronounced atrophy (Figs. 2, 3, 4, 5). Some of the larger arteries of the medulla show an acute suppurative inflammation which has resulted in aneurismic dilatations. The entire circumference of a

segment of the artery is involved. The walls of the diseased portions are densely infiltrated with polymorphonuclear and large mononuclear leucocytes. There is a striking resemblance to the lesions of periarteritis nodosa. A similar suppurative arteritis has been observed by us in subacute bacterial endocarditis. The diseased arteries in this kidney are responsible for some of the cortical atrophy; but the tubules that show a pronounced atrophy may be traced to glomeruli with closed capillaries (Figs. 2, 3, 4, 5). There is no necrosis of the tubular epithelium.

The glomeruli show very striking changes. The most conspicuous lesion is hyperplasia of the outer layer of Bowman's capsule with the formation of *epithelial crescents* (Figs. 6, 7, 8). This is often called extracapillary glomerulitis. It is found in 5 to 10 per cent of the glomeruli in different parts of the kidney. The crescent compresses the glomerulus and prevents the circulation of blood through its capillaries. A disuse atrophy of the glomerulus and its associated tubule follows. This type of lesion is frequently seen in human acute glomerulonephritis.

The most common glomerular lesion is *fusion between the glomerulus and the outer layer of Bowman's capsule* (Figs. 4, 5, 9, 10, 11). This results in partial or complete obliteration of the capsular space. In many of the glomeruli there is *swelling of the endothelium with closure of some of the capillaries* (Figs. 5, 8, 9, 10, 11, 12). Polymorphonuclear leucocytes are sometimes found in these closed capillaries (Fig. 11) and the swollen cells may show hyaline changes in their cytoplasm (Fig. 12). A true proliferative glomerulitis (increase in number of endothelial cells) is not a prominent feature. The proliferative lesion, it will be recalled, is the most common type in human glomerulonephritis. An occasional glomerulus shows large numbers of polymorphonuclear leucocytes in its capillaries and in the capsular space (Fig. 13). This is called *exudative glomerulitis.*

To summarize: Over half of the glomeruli show some form of injury which tends to interfere with their function. There are numerous instances of fusion between the tuft and the capsule of Bowman, and many glomeruli have a part of the capillary network occluded by swollen endothelial cells. Epithelial crescents are frequent and there are a few examples of exudative glomerulitis. The tubules belonging to the occluded glomeruli show a well-defined atrophy. The similarity to human glomerulonephritis is striking.

The lesion may be classified as an early stage of chronic glomerulonephritis. A convenient histologic distinction between acute and chronic glomerulonephritis is the presence of a well-defined tubular atrophy in the latter. The increase of urea nitrogen in the blood indicates a moderate degree of renal insufficiency, which is consistent with the histologic appearances.

No. 3. Large male. This experiment will not be reported in full in this paper since the monkey is still living. The experiment was begun March 1, 1924. Albuminuria has been present since May, 1924, and it has been severe since August. Recently (January, 1925) there has been a large amount of blood in the urine. Small injections, one to four weeks apart, are sufficient to keep up the severe albuminuria. The kidneys can still concentrate well, however, and the blood metabolites are not increased. There is no edema. The blood pressure is not known. The type of renal lesion present cannot be determined with certainty in the living animal.

Discussion

The intravenous injection of streptococci into fourteen monkeys produced severe renal lesions in five. There were two cases of severe nephrosis (Nos. 6 and 9), one of acute insterstitial nephritis (No. 12), one of glomerulonephritis (No. 2), and one of a nature not yet determined (No. 3), since the animal is still living. In the other 9 monkeys the organisms caused death without producing any serious renal injury. It is known that streptococcic infections in man may result, also, in glomerulonephritis, nephrosis, interstitial nephritis or an insignificant renal injury. No investigator has offered a satisfactory explanation of this varying effect of streptococci on the kidneys. It is known that in some cases at least of glomerulonephritis the endothelial cells of the glomerulus ingest the bacteria, and then undergo swelling and proliferate as a result; but bacteriemia usually causes nephrosis, not glomerulitis. There is some necessary factor other than the presence of bacteria in the blood stream that determines whether a glomerulitis will result. It is not known whether soluble bacterial toxins, without the bodies of the bacteria, can cause glomerulitis.

Three hypotheses may be offered to explain the origin of glomerulonephritis. (1) There is a specific etiologic agent — some special

strain of streptococci. (2) Many types of streptococci may produce the disease but only certain individuals are susceptible. (3) There must be repeated injury to the glomerular endothelium. Individual susceptibility and the strain of the infecting organism are perhaps not important. Our results thus far do not justify any conclusion as to which of these hypotheses is correct; but we are proceeding on the theory of repeated glomerular injury.

It may be objected fairly that our single successful experiment (No. 2) does not prove that glomerulonephritis is caused by streptococci, since it is possible that the disease may have developed during the course of the experiment independently of the experimental procedure. Other positive results must be obtained to establish the etiology of glomerulonephritis with certainty, but we feel that the solution of the problem is definitely nearer. An animal has been found in which the disease may develop, and there is at least a strong probability that the organisms injected produced the disease.

Conclusions

Intravenous injections in rabbits of several strains of streptococci from human sources produced in several instances nephritis of the spontaneous type (lymphocytic interstitial nephritis) but did not result in glomerulonephritis.

Similar injections in monkeys resulted in two cases of severe nephrosis, one of acute interstitial nephritis, one of glomerulonephritis, and one of a type not yet determined.

The case of glomerulonephritis was characterized by epithelial crescents, fusion of glomerular lobules to the capsule of Bowman, swelling of the endothelium with occlusion of the capillaries, and atrophy of tubules associated with occluded glomeruli.

Glomerulonephritis has been produced in one monkey by intravenous injections of streptococci.

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DESCRIPTION OF PLATES XLV–XLVIII

Plate XLV

Fig. 1. Monkey No. 12. Drawing. Acute interstitial nephritis. Large numbers of mononuclear leucocytes are found, widely distributed between the tubules in the cortex. They are most numerous around the small veins.

Fig. 2. Monkey No. 2. Photomicrograph, low magnification. Injured glomeruli with atrophy of their associated tubules.

Fig. 3. Monkey No. 2. Photomicrograph. Part of the field of Fig. 2 under higher magnification. One glomerulus is almost completely occluded by closure of its capillaries and fusion of the lobules to the capsule of Bowman. The glomerulus with the open capsular space shows collapse of its capillaries, and a thin epithelial crescent to which the glomerular lobules are partly adherent. The tubules show a marked atrophy.

Plate XLVI

Fig. 4. Monkey No. 2. Photomicrograph. In about half of the glomerulus the capillaries are closed by swollen endothelial cells, and this part is fused to a thin epithelial crescent. The tubule is markedly atrophied.

Fig. 5. Monkey No. 2. Photomicrograph. The capillaries are all closed by swollen endothelial cells and the capsular space is largely obliterated. The tubule is markedly atrophic.

Fig. 6. Monkey No. 2. Photomicrograph. A large epithelial crescent. The upper right-hand portion of the glomerulus shows occlusion of its capillaries, and it has become fused with the crescent and the capsule of Bowman.

Fig. 7. Monkey No. 2. An epithelial crescent. The glomerulus is anemic.

Plate XLVII

Fig. 8. Monkey No. 2. Photomicrograph. Higher magnification of a glomerulus shown in Fig. 10. There is a small epithelial crescent with fusion to the glomerulus in several places. In the central part of the glomerulus the capillaries are closed and there is some disintegration of the swollen endothelial cells.

Fig. 9. Monkey No. 2. Photomicrograph. Glomerulus showing extensive closure of capillaries and fusion to the capsular epithelium.

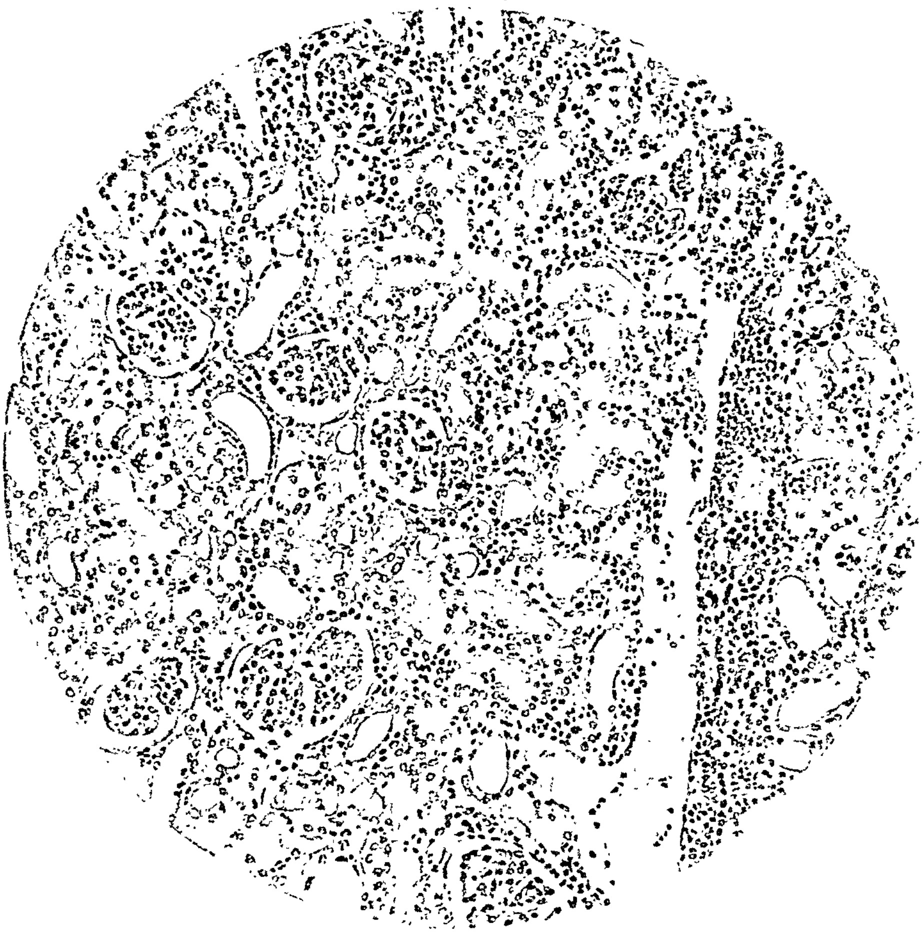
Fig. 10. Monkey No. 2. Photomicrograph. Two glomeruli showing inflammatory reactions. The left-hand glomerulus is shown under higher magnification in Fig. 8; the right in Fig. 11.

FIG. 11. Drawing of the lower half of the glomerulus shown in the left-hand portion of Fig. 10. There has been extensive proliferation of the endothelium with occlusion of the capillaries. In the upper left-hand portion several polymorphonuclear leucocytes are seen imprisoned in closed capillaries. This appearance is frequently seen in the proliferative type of human glomerulonephritis.

FIG. 12. Monkey No. 2. Photomicrograph. An enlarged glomerulus showing extensive swelling of the endothelium with capillary occlusion. A number of polymorphonuclear leucocytes are seen.

PLATE XLVIII

FIG. 13. Monkey No. 2. Photomicrograph. An example of exudative glomerulitis. There are a large number of polymorphonuclear leucocytes in the capsular space.



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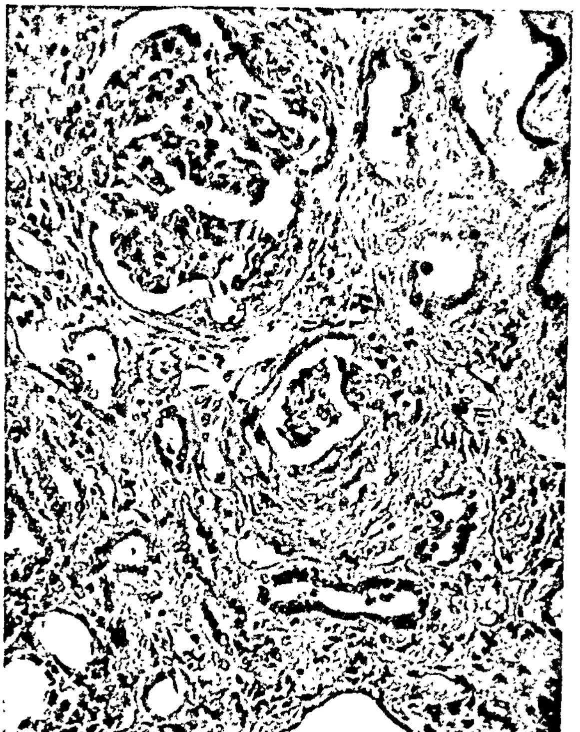

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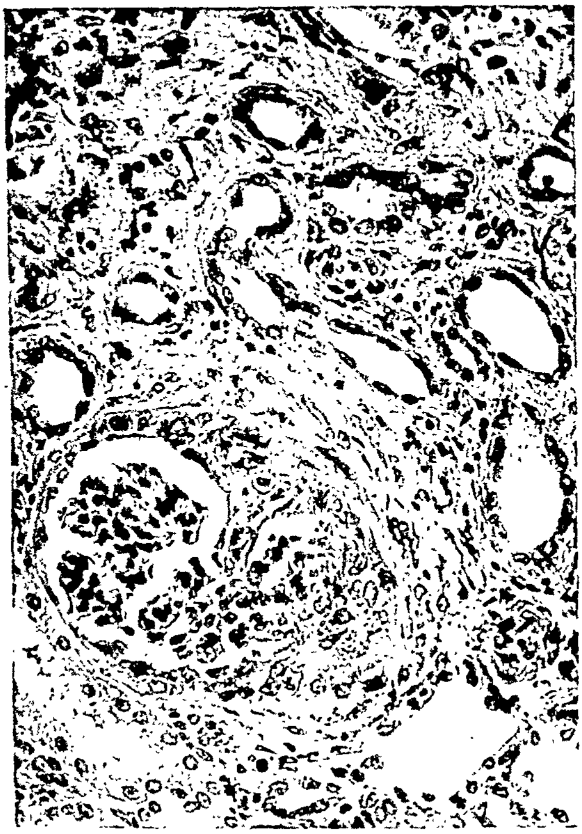
Bell, Clawson and Hartzell

Experimental Glomerulonephritis



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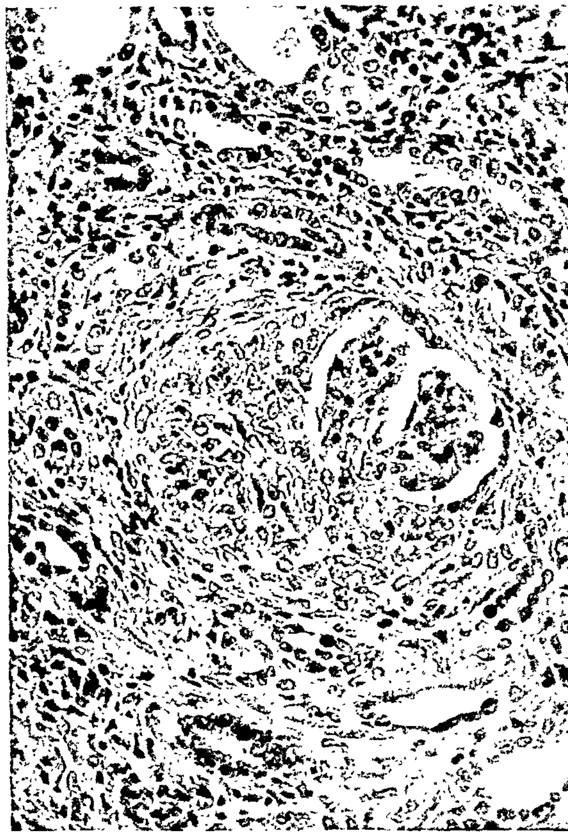

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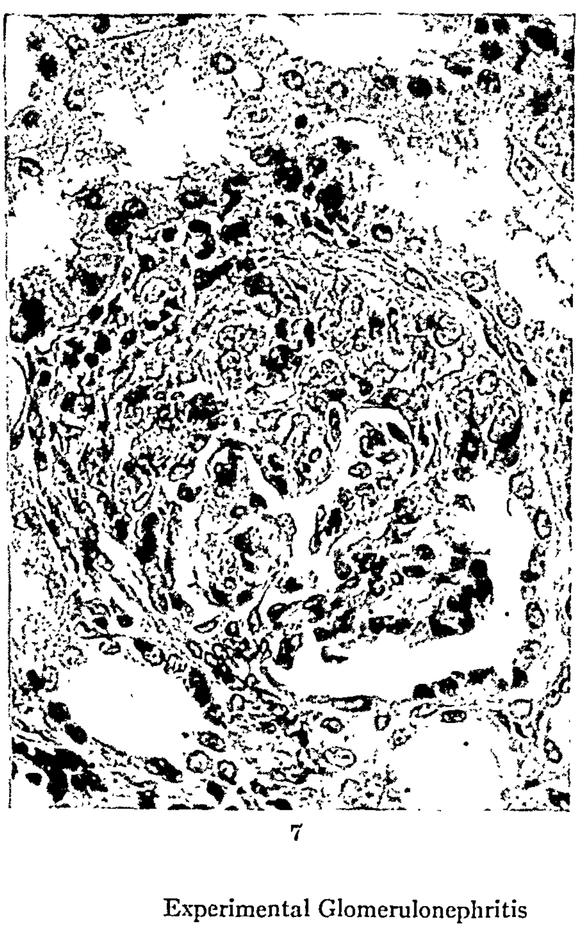

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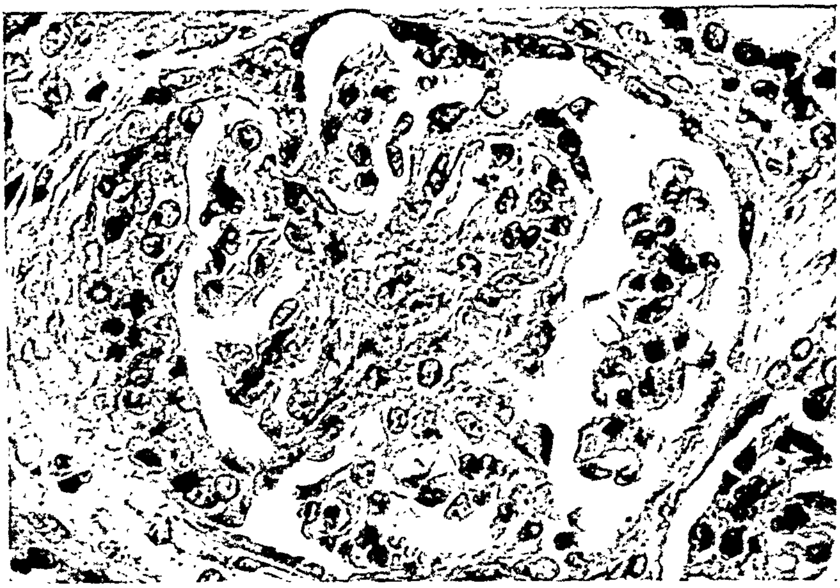

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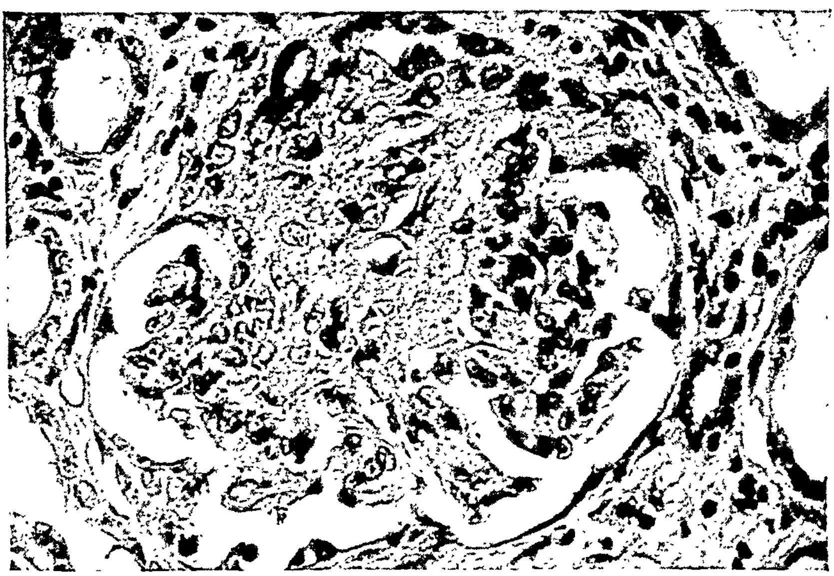

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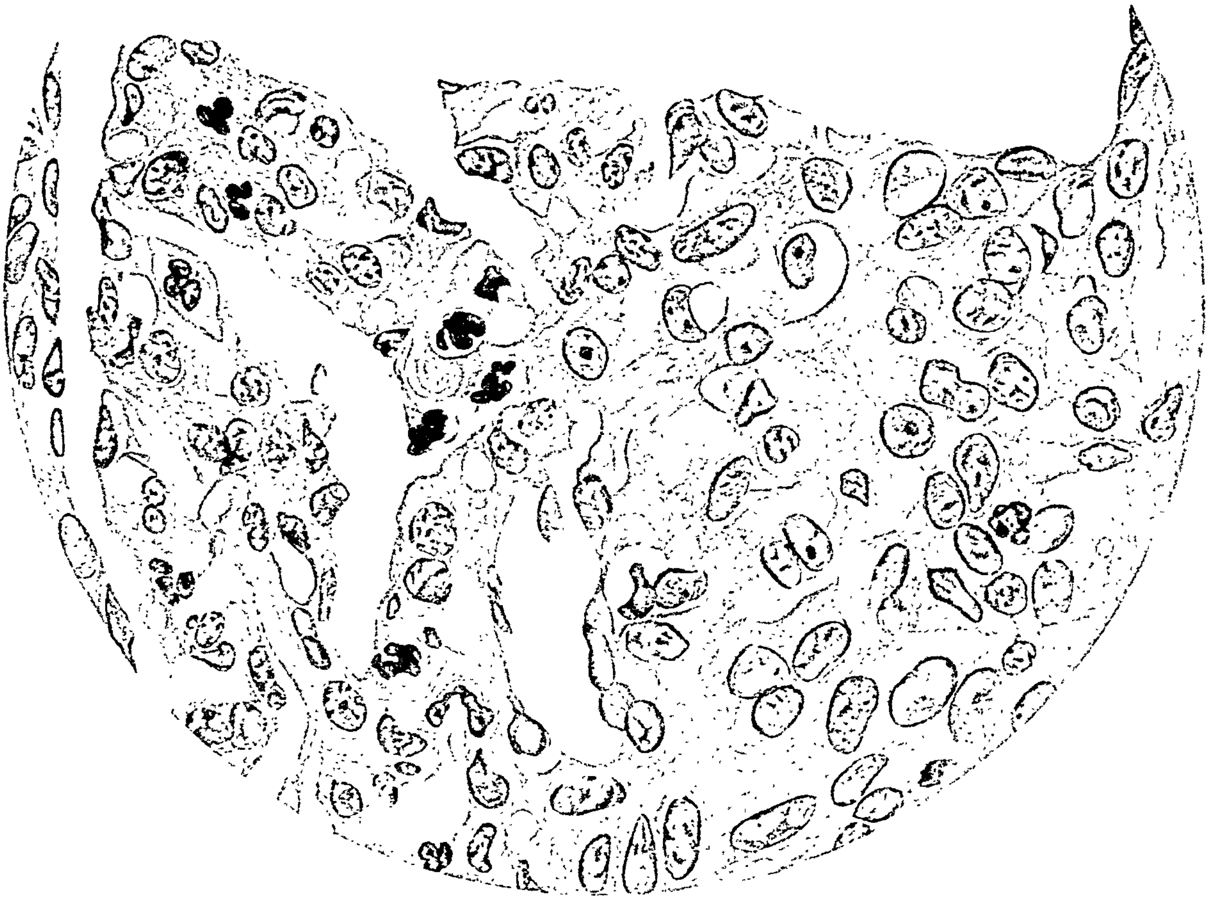

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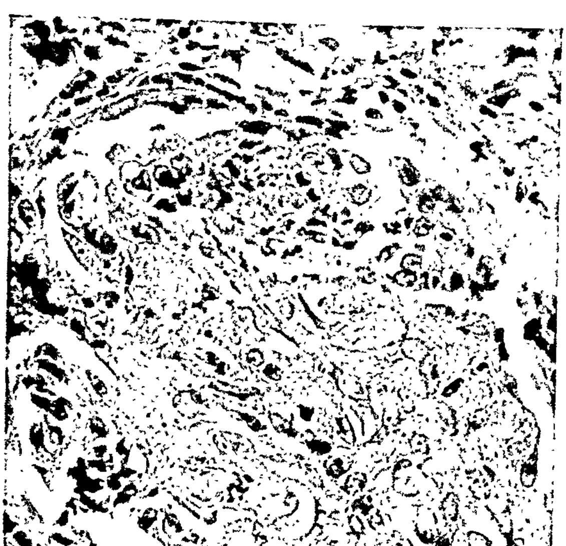

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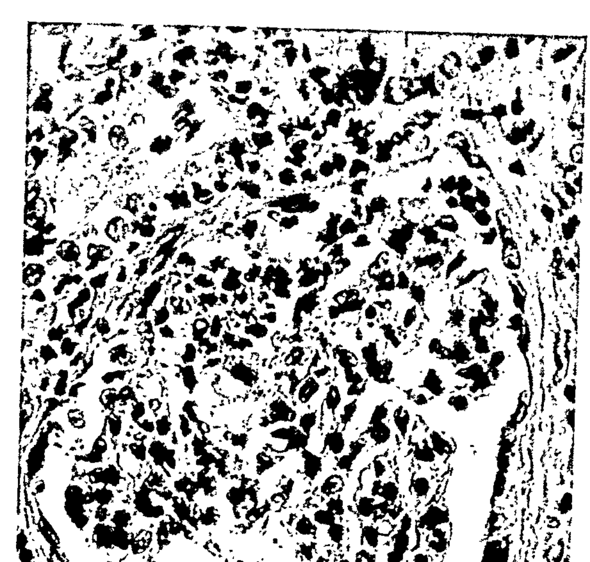

13

Bell, Clawson and Hartzell

Experimental Glomerulonephritis

THE FIBROMA OF THE ACOUSTIC NERVE *

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The cerebellopontile angle is an anatomical site in which certain neoplasms may develop, the glioma, the dural endothelioma and the acoustic nerve fibroma comprising the very small group of tumors (except for a rare metastatic growth) which have been found in this location. In 1917 Dr. Harvey Cushing [1] in his monograph "Tumors of the Nervus Acusticus" directed attention to the acoustic nerve tumor as a clinical and pathological entity. His own studies and those of Councilman, Mallory and Goodpasture lead also to the differentiation of a histologic picture characteristic of most tumors in the aforementioned locality, their origin usually being in the sheath of the eighth cranial nerve.

The greater percentage of acoustic tumors are unilateral. When bilateral they are usually an expression of a widespread affection of nerve sheaths, commonly known as von Recklinghausen's disease. Berggrün,[1] Raymond,[1] and Bassoe and Nuzum [2] have reported instances of bilateral tumors and others are on record. Dr. Cushing had none in his series of ninety-one acoustic tumors. This paper concerns three cases of cerebellopontile angle tumors which have come to autopsy in the pathological department of the Albany Medical College during the past three years. In one of them these neoplasms were bilateral.

It will not be attempted here to discuss with much elaboration the clinical aspects of these three patients. Their histories will be given in all available detail but the reader will be left to analyze for himself much of the clinical data. To the pathologist the anatomical and histologic characters of these tumors and their secondary effects are of greatest interest and furnish a rational explanation of the usual symptom complex. That tumefactions in the cerebellopontile recess, of eighth nerve origin, are productive as a rule of a pathognomonic syndrome is generally recognized; hence a brief presentation of that syndrome is apropos.

* Received for publication February 18, 1925.

"The acoustic tumors," Dr. Cushing states, "owing to the characteristic chronology of their symptoms, may as a rule be sharply distinguished from all other tumors of the cerebellopontile angle.... The symptomatic progress of the average acoustic tumor occurs more or less in the following stages: first, the auditory and labyrinthine manifestations; second, the occipito-frontal pains with suboccipital discomfort; third, the incoördination and instability of cerebellar origin; fourth, the evidences of involvement of adjacent cerebral nerves; fifth, the indications of an increase in intracranial tension with a choked disc and its consequences; sixth, dysarthria, dysphagia, and finally cerebellar crises and respiratory difficulties." For a bibliography of the condition the reader is referred to Dr. Cushing's work. Nothing of significance has been published since.

Case I. Hospital number 81547. Mrs. L. A., white, aged 46 years, admitted to the Albany Hospital, October 26, 1921, complaining of gradual loss of sight and hearing over a period of one and a half years, and difficulty in walking.

History: her general health has been good until eight years ago, when her head began to be spasmodically drawn to one side. Attacks of fainting during the first three weeks of illness and vertigo daily. Many attacks of blindness lasting from two to seven minutes and a general impairment of sight for one and a half years. Hearing has been poor past year with total deafness and tinnitus last eight months. Great difficulty in forming sentences although her memory seems good. Last two months dysphagia and marked dyspnoea. Lately her "flesh feels creepy."

Physical examination: The important findings are neurological. Eyes: O. D., pupil 4.5 mm., regular, movements slow. Media clear, disc swollen with edema, 8 diopters. Vessels large and prominent. O. S., pupil regular, 4.5 mm. diam., movements slow. Media clear. Disc swollen 8 diopters. Vertical pseudonystagmus when looking up. Diagnosis from ocular findings, choked discs, very great intracranial pressure.

X-ray examination: skull is unusually thick. The picture is unsatisfactory on account of the movements of the patient.

Summary of findings of Dr. LaSalle Archambault: Ocular excursion is restricted in all directions, but more markedly so upward and to the left. The pupils react actively to light. Facial and hypoglossal nerves negative. No paralysis in arms. Grasp excellent in both hands, but there is some evidence of asynergy and adiadokokinesis. There are similar findings in the legs. All reflexes more active on right side of body. A doubtful Babinski on right side. Many tests not attempted, owing to the marked deafness of the patient, which renders coöperation impossible. Impression: a lesion (possibly tumor) involving the dorsal segment of the pons Varolii at the junction of the superior cerebellar peduncle, the corpora quadrigemina and the posterior longitudinal bundle.

A decompression was advised but not done. She died April 15, 1922.

Autopsy was performed two hours post-mortem. The body shows much emaciation. On opening cranial vault and dura there is seen

a great increase of clear subarachnoid fluid. Cerebral cortex edematous and convolutions slightly flattened. On severing the tentorium cerebelli and lifting the brain upward and backward, two roughly oval, somewhat lobulated tumor masses are found lying one along each side of the pons and medulla and extending upward and forward to form indentations in the under surface of the lateral lobes of the cerebellum. The tumors have produced tremendous pressure upon and distortion of the brachia conjunctiva and the pons, so that the basilar artery takes a winding course over the structures normally in the midline. The masses have a rather smooth, grayish-pink surface and their attachments comprise chiefly their blood vessels and accompanying prolongations of arachnoid. On section each mass shows a grayish striated, very tough structure, not unlike a leiomyoma or dense fibroma. The eighth nerves are identified with difficulty, having broken away from the tumors to which they had apparently been attached. Gross examination of the other cranial nerves fails to reveal any other evidence of neurofibromatosis.

On section of the cervical cord, the central canal is found dilated to a diameter of two millimeters.

Microscopic examination. Sections of both tumors were stained with haematoxylin and eosin, phosphotungstic acid-haematoxylin, Mallory's aniline blue, and by Van Gieson's method.

The tumors are surrounded by a thin connective tissue capsule in which are dilated lymph and blood vessels. Predominating in the picture are areas of fibrous tissue running in irregular bands with a definite tendency of the nuclei of the cells to be arranged in a regimental manner. The fibrous zones alternate with smaller areas of loose edematous reticular tissue. All sections of the tumors are very vascular and in and about the reticular areas are many thrombosed vessels, some of which show hyaline degeneration of their walls. No neuroglia cells or fibers can be demonstrated in many sections examined. There is a general uniformity of size and staining of the spindle cells of the tumor in most sections, but in others the nuclei are large and deeply staining, like young cells and more rapidly growing. Mitotic figures are not found, however.

Case II. Hospital number 86470. Miss J. B., aged 22 years, entered Albany Hospital, September 21, 1922, complaining of double and blurred vision, transitory blindness and dizziness. Present illness began in 1921, with disturbance of vision and marked vertigo on change of position. For an indefinite period she has awakened each morning with occipital headache. Vision good until Sep-

tember 1, 1921, when there were sudden attacks of diplopia with blindness of two or three minutes' duration. Vision always worse in morning. No loss of color vision.

Neurological examination: N. I., no disturbance of smell; N. II, O. D., pupil 5 mm., regular and active. Media clear. Great elevation of disc, plus four diopters. Vessels of fundus of irregular caliber and in many places obscured. A few superficial retinal hemorrhages. Vision 20/20. Partial ptosis of lid. At rest pupils are 20° convergent. Coarse rapid lateral and vertical nystagmus and slight exophthalmus. O. S., has same fundus changes as O. D. Some muscle jerking and internal strabismus. Both pupils react to light and accommodation consensually. N. V, no motor or sensory disturbance. N. VII, normal. N. VIII, no tinnitus; vertigo on change of position. Air and bone conduction not tested. NN. IX, X, XI, XII are normal.

Great mental irritability during the past year. Well oriented as to time and place. Romberg, slight swaying. X-ray examination of skull reveals no evidence of tumor. Spinal fluid, September 19, 1922, globulin 4 plus, cells 8. Spinal fluid, October 18, 1922, globulin 3 plus, cells 6.

Diagnosis: unlocalized brain tumor.

Operation performed by Dr. Arthur H. Stein. A subtemporal decompression was done. On opening dura a considerable quantity of clear fluid escaped. There was considerable hemorrhage which was very hard to control. No gross evidence of tumor in cortex. Some of the more marked symptoms appeared to be relieved by the operation. On seventh day patient suddenly collapsed and from then on rapidly grew worse. Previous symptoms became more marked and a hernia cerebri very pronounced at the site of operation. There were much drowsiness, nausea and vomiting. Spinal fluid was under enormous pressure and some relief was gained by lumbar punctures. Patient gradually became weaker and more emaciated, due to the fact that she was unable to retain nourishment. Cerebral type of vomiting. Complete left-sided paralysis developed and lasted six weeks. Her condition gradually became worse with death on December 9, 1922.

Autopsy two hours post-mortem. There is a distinct bulging on the side of the head within the limits of the operative incision. This area is soft and fluctuant and scalp is adherent. The brain substance is bulging through the operative opening, being held by the temporal fascia and muscle.

On removal of the skull cap, the dura appears normal, except that it is so closely adherent to herniated area of the brain that it has to be removed with the brain. There is no edema or congestion of the blood vessels. The cerebro-spinal fluid is normal in amount and is perfectly clear. The longitudinal and transverse sinuses appear normal. The superior surface of the brain is symmetrical, except for the herniated area, which measures 6 × 8 cm. and projects 2 cm. above the general contour of the brain. This area is soft and spongy. The consistency of the brain is quite uniform over the entire convexity of both hemispheres. On removing the brain, the tentorium cerebelli is quite taut, especially on the left side, although there is no

bulging, but when it is cut on the left side, a yellowish tumor mass immediately protrudes through the opening, to which two or three good-sized blood vessels are running from the arachnoid.

The Inferior Surface of the Brain. There is a distinct cerebellar pressure cone. The under surface of the temporal lobes show slight roughening, as though adherent to the membranes. A rather soft and rubbery tumor mass occupies the region of the middle cerebellar peduncle, just anterior and superior to the left cerebellopontile angle. The visible portion of the tumor measures 3 × 3 × 2.5 cm. and on palpation extends back into the cerebellum into the amygdala and biventral lobe about 2 cm., so that from the inferior only a small portion of the lateral part of the lobuli centralis can be seen. The tonsil cannot be seen at all. It cannot be determined from a general examination whether the tumor mass is invaginated into the cerebellum or is an outgrowth from it. It occupies nearly the whole supero-inferior thickness of the cerebellum. It is invaginated into the pons, medulla and olives on the left side, distorting them markedly. The fifth nerve passes out over the tumor, between it and the brain stem and is pushed upward. There has apparently been no compression of the nerve. The entire brain stem has been pushed anteriorly and to the right. The tumor mass itself is roughly oval in shape, yellowish in color, smooth in outline and slightly firmer than the surrounding brain tissue. It is underneath the pia. There is much congestion of superficial blood vessels of the tumor and of the cerebellum.

Description of the brain after hardening in 4 per cent formaldehyde. The anterior tip of the right temporal lobe on its inferior surface has a moth-eaten appearance. There are numerous small areas in which there is an indentation of the cortex. The larger of these areas measures 0.5 cm. in diameter and extends down into the central white matter. There are numerous other smaller areas similar to the one described, but none of these extends through the cortex.

Frontal section at the level of the optic chiasm reveals considerable dilatation of the lateral and third ventricles. The recessus opticus is considerably dilated. The inferior and posterior horns of the lateral ventricles are involved in the dilatation.

There is considerable distortion of the convolutions on the right side at the bottom of the Sylvian fissure, due to the decompression.

The central white matter adjacent is much softened and has a pinkish tinge. At the level of the anterior commissure, this softened focus comes within 2 mm. of the dilated lateral ventricle.

On section there is seen to be marked asymmetry of the cerebellar hemispheres, the left being much larger. The left inferior cerebellopontile angle and the adjacent portion of the left cerebellar hemisphere are occupied by an encapsulated tumor mass measuring 3 × 5 cm. in its greatest diameters.

The tumor mass is only slightly adherent to the cerebellum but instead is invaginated into the left cerebellar hemisphere, pushing the cerebellar structures aside and compressing them. The tumor is surrounded by a delicate connective tissue capsule from the outer surface of which run fibrous tags attaching the tumor to the adjacent cerebellar surface and to the pons. The external surface of the tumor is quite irregular in contour, due to numerous hard elevations averaging 0.5 cm. in diameter. There are numerous dilated blood vessels on the external surface. The cut surface of the tumor presents a mottled, slightly gelatinous appearance. Numerous foci of hemorrhage and degeneration are scattered throughout.

There has been produced a deep excavation where the tumor has encroached into the left cerebellar hemisphere.

The cochlear division of the eighth nerve is much enlarged on the left side and runs on the superior surface of the tumor mass. It is very adherent to the tumor and apparently is lost in the substance of the tumor. The other cranial nerves show nothing noteworthy.

Microscopic examination. The sections are of a rather vascular neoplasm, composed of fasciculi of fibroblasts arranged in irregular bands and whorls, with in places a well-defined tendency toward regimentation of the nuclei, and of a loose, reticular tissue varying in amount from small isolated patches to predominance in some sections. As a whole, the reticular tissue is much less in amount than the fibrous tissue.

The fibroblasts have usually vesicular ovoid nuclei with one to three nucleoli, but there is variation with the nucleus being larger, more chromatic and irregular in outline. Fibroglia fibrils are conspicuous in phosphotungstic acid-hematin preparations and collagen fibrils form in several a fair amount of intercellular substance. Edema is very marked in the capsule and in large areas of tumor,

the fibroblastic tumor cells becoming very hydropic, so that little or no cytoplasm is in evidence. Contributing to the vacuolated "reticular" tissue is a moderate amount of fat and lipoid within cells of many shapes, but practically all of which can be traced back to the spindle tumor cells. Thrombosis and hyalinosis of blood vessels in and about the rarefied areas suggest the cause of the hydropic and fatty changes so much in evidence. Many of the blood vessels are closely packed with leucocytes, and there is perithelial or adventitial migration, chiefly of polymorphonuclear neutrophiles, about several of the smaller vessels.

Case III. Hospital number 94868. Mrs. L. J., a white American housewife, aged 44 years. Chief complaint is pain in the epigastrium, spine, and occipital regions. She has difficulty in recognizing distant objects with right eye and especially difficulty in seeing when fatigued. Hears poorly with right ear. Occasional attacks of tinnitus. Recently there has been pain in back of head and along entire spine, associated with a spastic condition of the muscles of the right side of face and right leg. In 1922 she began to be ailing, with severe left frontal headaches which were worse at night. Marked tinnitus aurium. In 1923 she had several attacks of faintness. Frequent severe attacks of abdominal pain requiring morphia. In November 1923, occipital pain developed and during the most severe attacks power to use arms and legs was lost. During one such attack the right angle of the mouth was drawn upward with twitching of all the muscles of the right side of the face and right leg. Profuse sweating of the right arm.

Physical examination: patient rather nervous and talkative. Both pupils react to accommodation. Bilateral horizontal nystagmus. Restricted movements of left eye toward left and of right eye toward right. Upward excursion of right eye limited. Bilateral vertical nystagmus but no downward limitation of motion. Some lagging of left eyelid. Bilateral choked discs. Optical fields suggest nasal and inferior contraction. Tongue deviates slightly towards left. Left angle of mouth elevated more than the right when upper lip is raised. Uvula deviates slightly to the right. Pain on pressure over the occipital nerve on the left side (Bing's sign). Upper extremities: reflexes slightly more active on the left and grasp is somewhat firmer on the left. Abdominal and ankle reflexes not elicited. No motor losses.

X-ray report, January 10, 1924: "No definite evidence of intracranial tumor."

January 15, 1924, hearing test:

	50	200	2000
Right ear	−	−	−
Left ear	+	+	+

First operation performed January 15, 1924, by Dr. Arthur Stein. The skull was trephined approximately 0.5 cm. below and to the right of the occipital protuberance. Dura was opened by a crucial incision exposing the cerebellum. The right lobe of the cerebellum was somewhat more prominent in the incision and showed venous congestion. To palpation the right lobe was more firm and

resistant, but no tumor mass could be felt with certainty. The patient's condition was such at this stage of the operation that it was decided to close the incision and do a second stage operation at a later date. A diagnosis was made of a right pontine tumor.

Second operation was done January 24, 1924. The old incision was opened and cerebellum uncovered. The right lobe of cerebellum showed marked bulging with evidence of necrosis of the outer layer. An attempt was made to elevate the right lobe of the cerebellum and reach the tumor beneath, but this could not be accomplished. The patient's condition would not permit further exploration, so the incision was closed and the patient was returned to the ward, where she died January 31, 1924.

Permission was obtained to explore the brain through the operative incision. This was done and a tumor approximately $3 \times 2.5 \times 2.5$ cms. was found, just above and anterior to the foramen magnum, and slightly to the right side. It was eroding the bone and had apparently involved the dura. The tumor mass was quite hard.

Gross description: The cerebellum, medulla and pons were removed, together with a tumor from the posterior fossa, through an occipital flap made for previous operations. The cerebellum is greatly lacerated, so that the connections of the tumor cannot be made out. The tumor is roughly spherical, faintly lobulated, measuring $4.2 \times 3.7 \times 2.5$ cms., inclosed in a delicate but tough fibrous capsule, which is interrupted over a very ragged surface 2.2 cms. in diameter, suggesting a point of attachment. It weighs 17 grams. The tumor is somewhat soft and homogeneous. Cut surface is smooth, rather translucent and bulges slightly from the capsule. On close inspection, fine fibrous strands are made out, frequently interrupted by irregular spots of opaque yellow. Many punctate hemorrhages mottle the surface.

Microscopic examination. The tumor is surrounded by a thin fibrous capsule, in which are blood and lymph vessels. The neoplasm is essentially fibrous throughout, with fairly abundant vascular supply. Large foci have undergone anemic necrosis, due to thrombosis of many large thin-walled blood vessels, which are also greatly dilated. The type cell is spindle and of varying size, from narrow, typically mature, ordinary fibroblasts, to larger, shorter cells, with nucleus of deeper tinctorial reactions and of much variety as to size and shape. No mitotic figures are found. The tumor cells are arranged in wavy bands, large tracts and in coarse whorls, the nuclei in places being arranged in so-called regimental or palisade manner. Fibroglia

fibrils are well demonstrated by specific connective tissue stains. There are many portions, usually adjacent to necrotic tissue, which present a picture of less marked degeneration, but with more vacuolation of cytoplasm and the formation of large, clear spaces quite like the "reticular" zones described in Cases I and II. In the vicinity of foci of necrosis or degeneration are groups of large, pale phagocytes, filled with lipoid.

Discussion

The acoustic neurofibroma had possibly been described as early as 1777 by Sandifort of Leyden,[1] but it is not certain absolutely that he was not dealing with a primary meningeal tumor. Jean Cruveilhier[3] in 1835 gave a masterly description of the clinical and gross pathologic findings in a true case, with drawings which leave no doubt as to the nature of the growth. Toynbee[4] in 1853 and Stevvens[5] in 1879, published observations of undoubted acoustic tumors. Sternberg[1] in 1900 reported four cases of his own and reviewed the literature. About this time experimental studies gave more accurate means toward the detection of cerebellar and other posterior brain lesions, so that from then on acoustic or "recess" tumors were more frequently recognized. For a complete bibliography to 1917 one should consult Dr. Cushing's monograph.

In a series of 468 verified brain tumors observed by Cushing to February 1, 1917, 134 were lesions in the posterior fossa involving the mid and hind brain; 56 of these were extra-cerebellar tumors and of these 30 came from the acoustic nerve. Six per cent, then, of all brain tumors were derived from the eighth cranial nerve. In a personal communication to the writer, Dr. Cushing brought the cases up to a very recent date; in a total of 1035 verified brain tumors there were 91 of these acoustic tumors or 8.8 per cent.

In Cushing's first series, eighteen were in females and twelve in males. The figures of Henschen[6] and Bruns,[1] on the other hand, show a slight predominance of males. The three cases here recorded were all in women. It is thus seen that there is no very definite sex predisposition to these lesions.

The first symptoms of acoustic tumor are often not remembered by the patient and hence the real date of onset of the disease cannot be told with accuracy. In Cushing's first series he found that 34

years was the average approximate age of onset. Our three cases were respectively 46, 44, and 22 years old at the time of entrance to the hospital for the alleviation of distressing symptoms.

Bilaterality in acoustic tumors is very rare. Dr. Cushing has had no instance in his large experience, although he has suspected it in several patients. Berggrün [1] and Bassoe and Nuzum [2] have reported each a case, the former in a child of eleven years with inherited generalized neurofibromatosis, the latter in an adult with neurofibromatosis. Raymond's [1] case apparently was without generalized nerve involvement as was our Albany case. Henschen [6] in 1915 compiled a large series of acoustic tumors, 245 unilateral and 24 verified bilateral tumors, the latter usually but not always identified with widespread neurofibromatosis.

The acoustic neurofibroma is said to originate from the perineurium of this nerve and usually in the distal portion of the nerve within the auditory canal. There may result through pressure absorption a dilatation of the porus acusticus internus. In the three Albany cases there was no helpful X-ray finding, but they were not studied sufficiently from this point of view to rule out changes in the porus. The post-mortem examinations also did not pay specific attention to this detail.

The cerebellopontile angle tumors have been known by many names, the long list only accentuating the lack of unanimity among pathologists as to the origin of the type cell and the significance of various other cells and their processes. Some of the terms found in the literature include the following: steatoma, fibroma, sarcoma, neuroma, glioma, endothelioma, sarcoma fusicellulare, neuroma fasciculare, neurofibroma, endotheliosarcoma, gliomes polymorphes angiomateux, neurinoma, and fibroblastoma.

Throughout this list there runs fairly consistently a vein of recognition of the outstanding feature of these tumors, i.e., their essentially fibrous nature. That there should be at times confusion with the dural endothelioma is easily understood because of the extracerebellar position and the similar texture of the two kinds of tumors. Mallory [7] only a few years ago concluded that the dural endothelioma was really a fibroblastoma because the tumor cells were capable of forming fibroglia and elastic and collagen fibrils. An association of true meningeal tumors with acoustic tumors has been observed by several writers since Wishart [8] first noted it in 1822.

Glia fibrils are known to occur in these tumors at times but usually they are hard to find. Their presence would seem to be largely accidental and more in the nature of inclusions or congenital glial rests, although in view of their occasionally having been seen it is perhaps natural that the diagnosis of glioma or gliosarcoma has been made.

What, then, is the characteristic appearance of these acoustic tumors? They are encapsulated, somewhat lobulated, more or less vascular fibrous tumors which vary in size from a few millimeters to as much as seven centimeters. The blood vessels are thin walled and prone to small hemorrhages so that hemosiderin deposits may be present. The vessels are also inclined to thrombosis and hyalin change with consequent focal necrosis, lipoid phagocytosis, and degenerations, the latter including probably the so-called reticular tissue. The type cell is a narrow spindle cell of fibroblast morphology but with slight variation from the normal tinctorial fibroglia and collagen reactions. The cells are arranged in interlacing bands and whorls, often with a striking parallel or regimental formation of the nuclei, a condition not infrequently seen in peripheral neurofibroma and even in leiomyoma. In some of these acoustic tumors this nuclear parade reminds one forcibly of the feather marking of a Barred Plymouth Rock fowl. The explanation of this is unknown. Mitotic figures are rarely seen.

There is also another quite constant feature, the zones of "reticular" tissue. These zones consist of cells with round and rather deeply staining nuclei and scant cytoplasm, lying in a much vacuolated matrix. There is often much free fat and lipoid in these foci and also hydrops of the cells. At first glance one notes a certain resemblance to glioma but neuroglia fibrillae are usually absent and the processes give, albeit often not entirely developed, characteristic staining reactions for fibroblasts or their products. Elastic fibres are not present except those derived from the blood vessels. The association with such foci of thrombosed or hyalinized blood vessels suggests that the "reticular" tissue is essentially the result of anemia of a portion of the tumor which formerly was probably of a dense fibrous nature like the rest of the growth. Isolated nerve fibers of acoustic nerve origin are rarely met with nor is there usually anything resembling a ganglion cell such as has been noted by Councilman in one of Cushing's cases. No corpora amylacea are present.

Summary

1. Three cases of verified tumors of the cerebellopontile angle are presented. In one instance the tumors were bilateral and of practically the same size.

2. These tumors by virtue of their location give rise to a characteristic clinical picture.

3. The four tumors in the three cases reported are of almost identical histology and agree essentially with the descriptions of the tumors of the nervus acusticus as described by Cushing, Mallory, Councilman and Goodpasture.

4. They are fibromas with, however, slight deviation from the staining reactions of fibroblasts in tumors elsewhere. Their origin is apparently from the perineurium of the acoustic nerve and they have many points of resemblance to peripheral neurofibromata. A regimental or palisade arrangement of nuclei is practically always present in some degree. The so-called reticular tissue is probably the result of degeneration due to hemorrhage or thrombosis or other vascular injury.

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DESCRIPTION OF PLATES XLIX–LII

Plate XLIX

Fig. 1. Case I, photograph of the base of the brain showing bilateral acoustic tumors in the cerebellopontile angles, producing marked pressure changes in pons and cerebellum.

Plate L

Fig. 2. Case II, photograph of the acoustic nerve tumor deeply embedded in the right lobe of the cerebellum.

Fig. 3. Case II, a vertical section through the cerebellum, showing the tumor to be well encapsulated but causing great pressure effects upon cerebellum, cerebellar peduncle and pons.

Plate LI

Fig. 4. Case I, a detail showing the parallel alignment of nuclei.

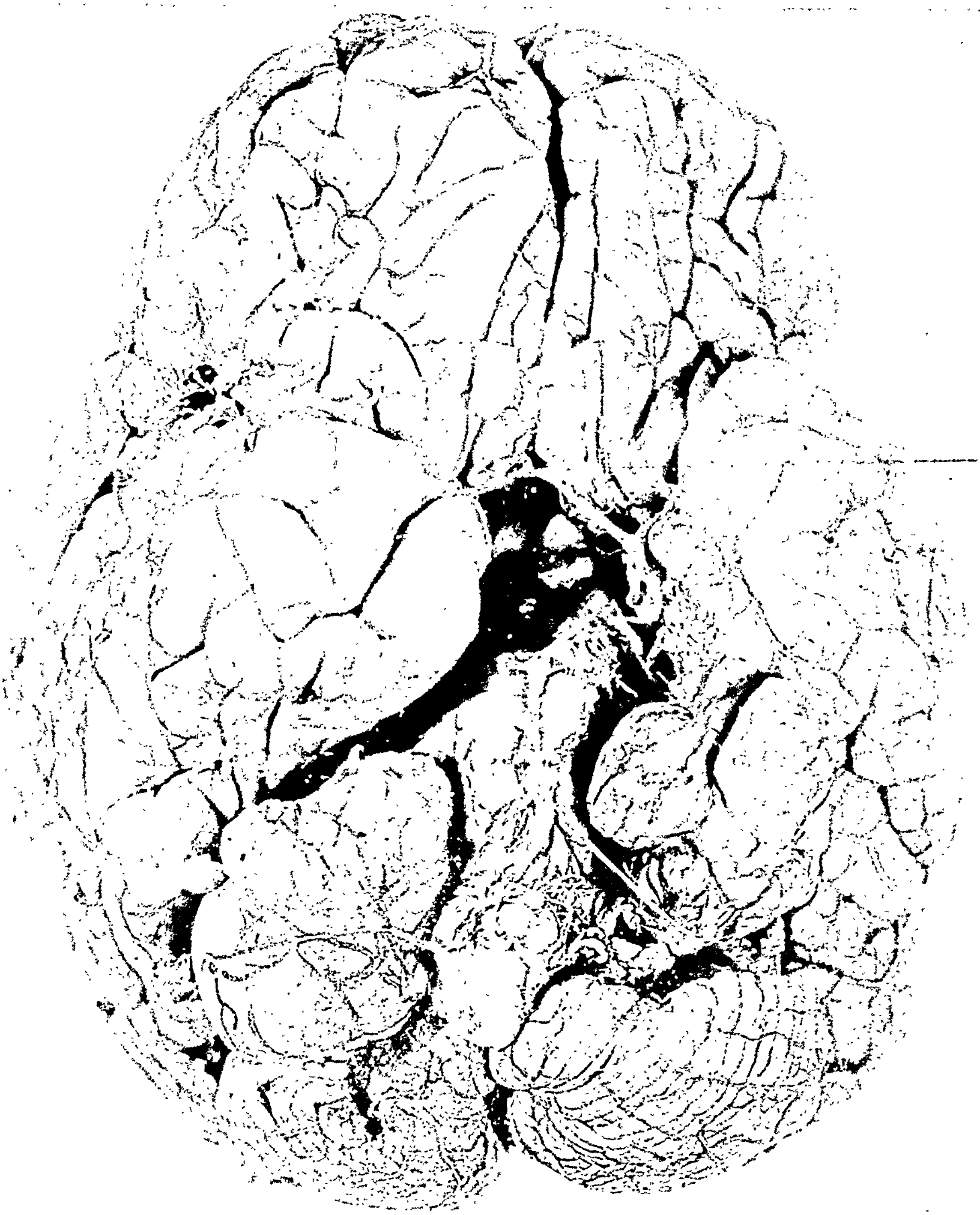
Fig. 5. Case II, a zone of "recticular" tissue (edematous and much vacuolated) with a thrombosed capillary and several capillaries with hyaline thickening of their walls.

Plate LII

Fig. 6. Case II, showing the fibrous nature of the tumor and in several places a parallel or regimental arrangement of nuclei. In the upper right-hand corner is a small focus of "reticular" tissue.

Fig. 7. Case III, parallel nuclear formation and an abundance of fibroglia.

Note. The photomicrographs were made from haematoxylin-eosin stained sections except Fig. 7, which was a phosphotungstic acid-haematin preparation.



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Jacobson Fibroma of the Acoustic Nerve

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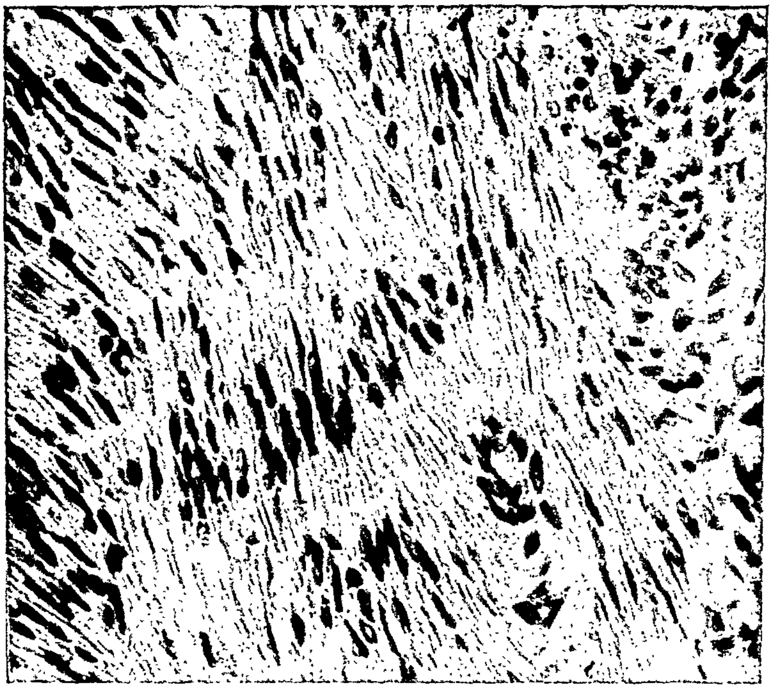

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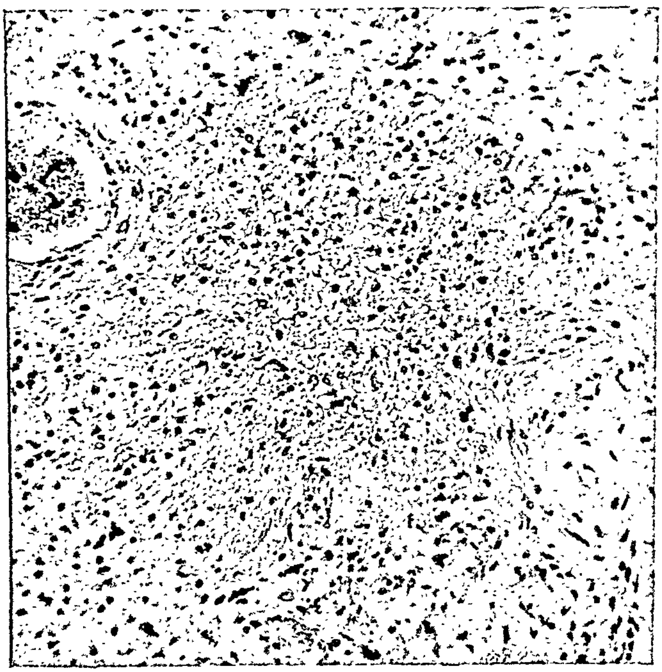

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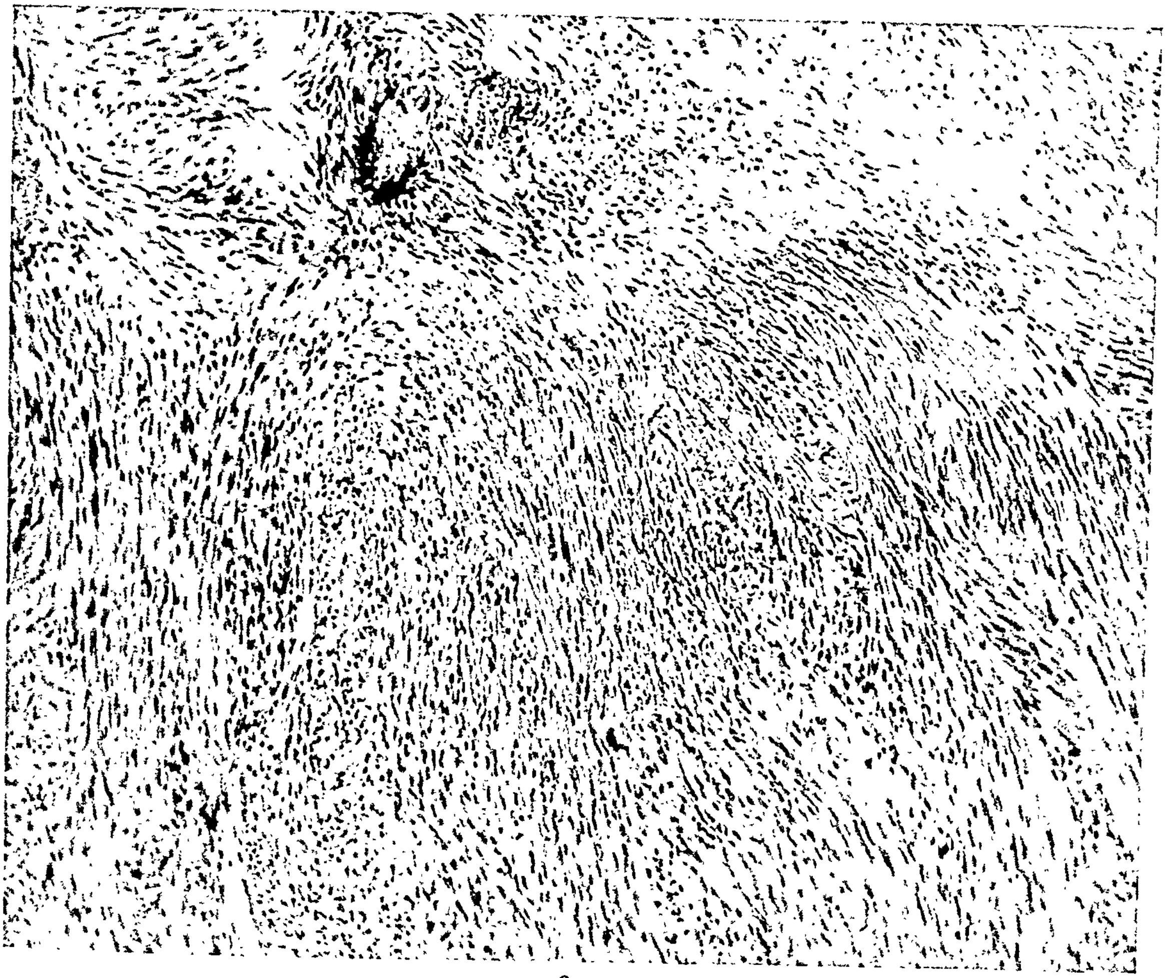

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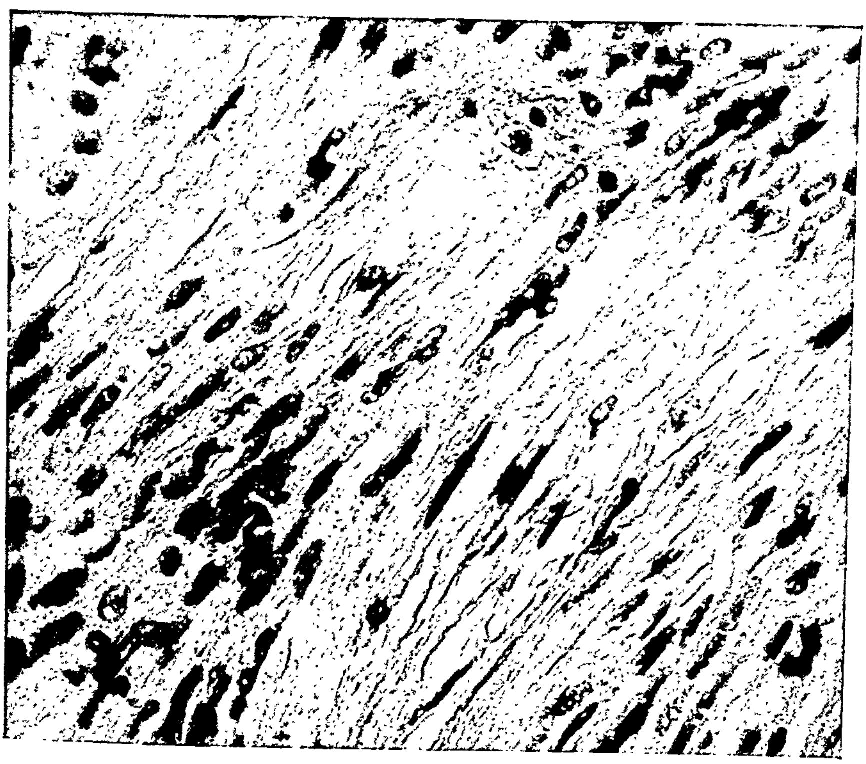

7

Jacobson

Fibroma of the Acoustic Nerve

THE PATHOLOGY OF THE HYPOPHYSIS

II. LYMPHOCYTIC INFILTRATION *

J. P. SIMONDS AND W. W. BRANDES

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In a study of more than 200 hypophyses in serial sections, 21 cases were encountered that showed areas of lymphocytic infiltration. The type of infiltration here reported is quite different from that which occurs about gummas and tubercles in the hypophysis, and there is little in the literature concerning it. The lesion was mentioned by one of us in a previous paper.[1] Pirone[2] observed lymphocytic infiltration in the hypophysis in hydrophobia; and M. Simmonds[3] and Berblinger[4] mentioned its occurrence in the vicinity of metastatic tumors in the posterior lobe. The 21 cases in our material have been studied with the hope of learning something of the nature and significance of the lesion. It has not been possible to investigate satisfactorily its relation to clinical symptoms because many of the patients from whom these hypophyses were obtained suffered sudden deaths and no anamnesis was obtainable.

It has been possible to divide these 21 cases into four groups as follows:

Group I. Those in which the area of lymphocytic infiltration was in immediate relation to one or more masses of colloid, either free in the tissues or inclosed in a space lined with epithelium.

Group II. Those in which the mass of lymphocytes was closely associated with one or more blood vessels and often resembled a potential lymph gland.

Group III. Those in which the lymphocytes diffusely infiltrated the tissues involved, without relation to blood vessels or colloid.

Group IV. Those in which the infiltration occurred as perivascular cylinders.

Border-line cases between Groups I and II occur. Without going into detail in individual cases, the following summary will suffice to present the essential features characteristic of each group.

* Received for publication February 24, 1925.

Each of the eleven cases in Group I showed one or more areas of lymphocytic infiltration in the middle lobe of the hypophysis. The location of these masses was rather constant. They lay very near the border line between the middle lobe and the neurohypophysis, frequently extending into the latter, and usually situated well laterally from the median plane. Furthermore, the infiltrating cells did not, in general, form a sharply circumscribed mass but gradually lost themselves in the surrounding tissues. In most of the cases the supporting tissue of the mass was the ordinary connective tissue which the cells had infiltrated. In two cases, however, the area suggested the follicle of a lymph node in appearance. The mass of infiltrating cells in almost all cases was quite near a small blood vessel. In all except one case it was intimately associated with colloid material, either lying free in tissue spaces, or inclosed in small "cysts." These cysts were more or less completely lined with low columnar or cuboidal epithelium and contained colloid that was vacuolated and strewn with desquamated epithelial cells. In one case, the desquamated cells had fused into a sort of syncytial mass containing a dozen or more nuclei. In two cases, plasma cells were present, either in the edge of the area of infiltration or nearby in the surrounding tissues. However, collections of colloid in the hypophysis are not always associated with lymphocytic infiltration.

The ages of the patients of Group I ranged from 20 to 50 years. All except two were 35 or more years old. There were only two women in this group. The pathologic changes found in other organs of the body varied greatly. One patient died from mitral stenosis, another from tumor of the brain, another from hemorrhagic encephalitis, the fourth from rheumatism and an ulcerative colitis of of undetermined origin, and the fifth from chronic meningitis three months after fracture of the skull. The remainder of the patients of this group died as a result of trauma. Microscopic examination of sections of different organs for areas of lymphoid infiltration was without result except in the case of the liver from the patient with ulcerative colitis.

Group II includes six cases. In these the supporting tissue in the areas of lymphocytic infiltration was typical reticulum. In all six cases, with possibly one exception, cells were present which resembled those that make up the germinal centers of a lymph node. These cells were not massed together as in a typical germinal center,

but were diffusely scattered among the lymphocytes in the central portion of the area. The infiltrations in this group were rather sharply circumscribed but did not possess a capsule. No plasma cells were found in or near the lesion in any case. The mass of lymphocytes in typical instances was not associated with colloid, either free or inclosed in epithelium-lined spaces. They did, however, have a rather constant and characteristic relation to blood vessels.

The posterior lobe of the hypophysis extends downward and forward into a cup-like depression in the anterior and middle lobes. In the median plane, its tip is separated from the middle lobe at the bottom of the depression by a mass of connective tissue in which is a plexus of blood vessels numbering from 5 to 20 in different sections. The infiltrating mass of lymphocytes in this group usually lies within this plexus between adjacent blood vessels. There is no tendency for the lymphocytes to surround one or more of the vessels, hence this lesion is distinctly different from perivascular infiltration.

This group contains four men and two women. The youngest patient was 12 years old, the ages of the others ranged from 35 to 55 years. One died of generalized miliary tuberculosis with tuberculous meningitis; another with chronic ulcerative tuberculosis of the lungs; one from chronic diffuse nephritis; a fourth from cerebral hemorrhage. All of these patients, except the 12-year-old girl, had varying degrees of arteriosclerosis.

In Group III (with two cases), the lymphocytic infiltration occurred in the anterior lobe. In one instance it involved the middle lobe and extended forward into the anterior lobe; in the other, it was limited to one lateral half of the glandular portion of this organ, which showed also a chronic hypophysitis. The infiltration in each case was diffuse, more so in one case than in the other. No plasma cells were found. One of these patients was a young woman, 20 years of age, who died of chronic alcoholism and exposure. The other, with the chronic hypophysitis, was a middle-aged man who died of lobar pneumonia.

In each of the cases of Group IV the infiltration was perivascular and located in the posterior lobe, usually near the capsule. The first of these patients suffered from an acute streptococcus meningitis, but the cylinders of perivascular infiltration were composed of lymphocytes. The second patient was an elderly man with marked chronic

myocarditis and arteriosclerosis of the coronary arteries, who had, two weeks before death, suffered an injury to the head which caused a slight hemorrhage in the pia and superficial part of the brain. The infiltrating cells in this case were different from those in any other case of this series, in that they resembled transitional or endothelial leucocytes.

Discussion

The nature and significance of the lesion in these 21 cases is perhaps different for each of the four groups. While lymphocytic infiltration in the hypophysis of the type here described does not appear to have been previously reported; it has been described in the thyroid and adrenals. M. Simmonds [6] found areas of lymphoid infiltration in 20 per cent of normal thyroids and in 80 per cent of thyroids in exophthalmic goiter. In this gland the lesion may appear in any degree from a small accumulation of lymphocytes to large areas with germinal centers and resembling in all essential respects, a follicle of a lymph gland. MacCallum [7] thought that lymphoid infiltration of the thyroid in toxic goiters was due to the action of toxic or injurious substances. M. Simmonds [8] believed that the accumulations of lymphocytes in the thyroid represent a reaction caused by the effects of an abnormal secretion. Kocher [9] observed areas of lymphocytic infiltration in both exophthalmic and adenomatous goiters, both in the adenomas and in the adjacent tissues. They were not, however, characteristic of toxic goiters because they were occasionally found in normal thyroids and in simple strumas. In most cases the lymphocytic infiltration was not related to blood vessels; in a smaller number, it was perivascular. The accumulations of lymphocytes were most frequent in those sections in which the acini showed absence of colloid and multiple layers and desquamation of the epithelium, and in areas of atrophy of the thyroid tissue. Kocher considered lymphocytic infiltration as an expression of chemical changes in the thyroid. V. Gierke [10] thought that lymphoid infiltration in exophthalmic goiters was associated with status thymico-lymphaticus. With this view both Simmonds [8] and Kocher [9] disagree.

Paunz [11] and others have described round-cell infiltration in the adrenals. Paunz thought that this condition was a reaction to a chronic infection or to a chronic intoxication which might be of

endogenous origin. This would partially explain the more frequent occurrence of this lesion in persons of middle age or older.

In as much as lymphocytic infiltration in the hypophysis occurs chiefly in or near that part of the gland which contains colloid, it would seem that this lesion in the hypophysis might be somewhat similar in nature to the similar condition in the thyroid. In Group I of our series of cases the similarity is all the more apparent because in this group the areas of lymphoid infiltration were definitely related to the presence of colloid. When the colloid was free in the tissue spaces, as in Case 1, it might stimulate the accumulation of lymphocytes just as any other foreign body might do. In those cases in which the colloid was inside an epithelium-lined cavity, it appears to be abnormal because: (1) it is vacuolated; and (2) contains many desquamated epithelial cells, as in the thyroid in many cases of toxic goiter; and (3) the epithelial lining of the "cyst" which contains the colloid is often abnormal in arrangement and in the number of layers of its lining cells. Whether, in these cases, the colloid is actually changed in quality it is not possible to determine from the data at hand. This possibility is suggested, however, by the fact that not all colloid-filled spaces are associated with round-cell infiltration.

None of the patients in our series showed any evidence of status thymico-lymphaticus. In only one was the thymus still present, and this was a young man 20 years of age. It is our opinion, therefore, that the lymphocytic infiltration in Group I is due to some local cause, possibly some change in the character of the secretion of the middle lobe. The presence of occasional plasma cells lends support to this view.

There is nothing about the accumulations of lymphocytes in the cases of Group II, on the other hand, that indicates a local cause. They occur, in their characteristic form, in a plexus of blood vessels, and are not directly related to the presence of colloid. The larger ones resemble an imperfect follicle of a lymph node: (1) in their rather sharply marked boundaries; (2) in the arrangement of the component lymphocytes; (3) in the reticular type of supporting tissue; and (4) in the presence of large cells with vesicular nuclei similar to those in the germinal centers of lymph glands. In other words, the areas of lymphoid infiltration in this Group are essentially like the lymphocytic accumulations in the lungs described by Arnold,[12] and in the

liver by Ribbert,[5] Marcuse,[13] and others. None of the patients in this group showed any indications of status thymico-lymphaticus. Three of them had tuberculosis, another, chronic nephritis; and two had marked arteriosclerosis. Hence it is possible that in this group of cases the occurrence of masses of lymphocytes in the vascular plexus of the hypophysis may be related to chronic infection or to chronic intoxication, as Paunz[11] thought was the case in the adrenals.

The presence of this type of lesion in the hypophysis is significant in another respect. Such accumulations of lymphocytes in other organs, as pointed out by Ribbert[5] in the case of the liver, are intimately associated with lymphatics. The presence of these areas of lymphocytic infiltration is suggestive evidence, therefore, contrary to the opinion of Thaon,[14] that this gland does possess lymph vessels.

Of the two cases in Group III, the lesion is easily explained as a part of a chronic inflammatory process in the anterior lobe of the hypophysis. The first case of this group, however, is the only one of its kind in this series, and we are unable to offer any plausible explanation of its presence.

In Group IV the infiltration is perivascular. In the first case the infiltrating cells were lymphocytes and the lesion was evidently an extension along blood vessels of an inflammatory process in the meninges. Simmonds[15] mentions involvement of the hypophysis by perivascular extension in cases of tuberculous meningitis. The lesion in the second case was characterized by an accumulation of transitional or endothelial leucocytes about a blood vessel. It is not possible at present to suggest any explanation as to its cause or nature.

While these 21 cases fall rather easily into the four groups outlined above, no symptoms or physical findings were common to a sufficient number to indicate the existence of a clinical entity corresponding to the histologic lesion. The study of the patient and the post-mortem examination of the body were not made with this distinct object in view, however.

It is perhaps justifiable to consider this round-cell infiltration of the hypophysis as a definitely pathologic process, in the nature of a reaction to some sort of stimulus. It will be remembered that Simmonds[8] found lymphocytic accumulations in 20 per cent of thyroids that were apparently normal. We have found this lesion in approximately 10 per cent of all of the hypophyses examined. Too little is

known of the pathology of this gland to state with certainty whether many of those examined were normal or abnormal. However, we are inclined to consider the lesion in Groups I, III, and IV as a reaction to some local stimulus or irritant, and in Group II to some extra-hypophyseal stimulus. To what extent this process or the condition which caused it interferes with the function of the hypophysis cannot now be stated.

Summary

1. Twenty-one cases of lymphocytic infiltration of the hypophysis, a lesion that does not appear to have been previously described in detail, are reported.

2. The process occurs most frequently in the middle lobe, less commonly in the anterior and posterior lobes.

3. It has been found in about 10 per cent of all hypophyses examined, and has been compared with the similar condition sometimes found in the thyroid and adrenals.

4. In the hypophysis the lesion appears in four distinct forms, three of which are believed to be the result of reaction to a local stimulus, the other to be associated with chronic infection of some distant organ or with some chronic intoxication.

5. The data available are insufficient to justify an opinion as to the effect of this change on the function of the hypophysis.

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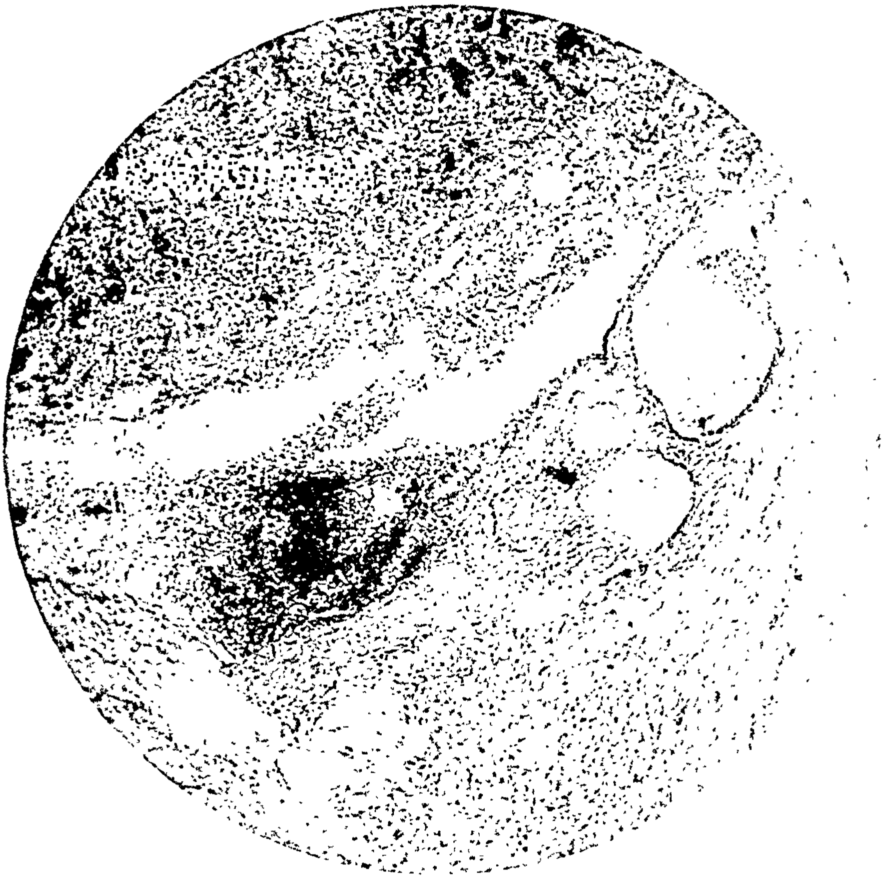
DESCRIPTION OF PLATE LIII

FIG. 1. Area of round-cell infiltration associated with colloid. Immediately below the area of infiltration is an acinus in which the epithelium is desquamated. X 70.

FIG. 2. Round-cell infiltration adjacent to blood vessels (at the left) and to abnormal acini (at the right). X 130.



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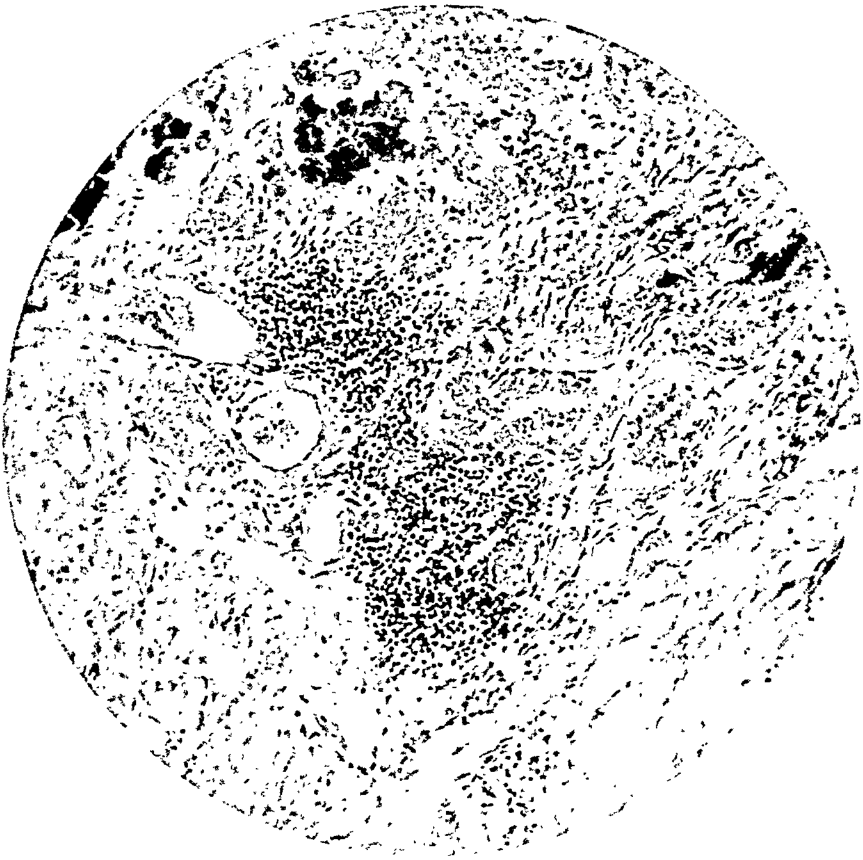

2

AN ADENOCARCINOMA RESEMBLING THE THYROID GLAND *

Wm. H. Feldman

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Neoplastic formations in the lower animals in tissues which resemble the pattern normal to the thyroid gland are of infrequent occurrence. The veterinary pathologist occasionally sees heterotopic manifestations of various organs but for the most part the tissue elements comply with the requirements of the architectural design characteristic of the tissue or organ duplicated and seldom exhibit a progressiveness of such nature as to qualify as a tumor.

In the following we have a structure that is unusual enough in its design and location to warrant description, although the factors bearing upon its etiological aspects will probably remain a matter of conjecture.

Case Report. In October 1923, Dr. G. G. Feldman, veterinary inspector in the United States Department of Agriculture, Bureau of Animal Industry, stationed at Spokane, Washington, forwarded to our laboratory a tumorous mass with the following history: A five-year-old grade cow was presented for slaughter. A spherical formation about six inches in diameter was observed attached under the skin over the muscles of the neck lateral and just posterior to the first cervical vertebra. After slaughter the mass was removed and placed in formalin fixative for future microscopic diagnosis and study.

Macroscopic appearance of tumor. The tumor was spherical in shape about six inches in diameter, fairly firm in consistency and of a flesh color which gradually faded to a whitish gray. Over the unattached surface under the skin a capsule was present. The surface was smooth without ulceration and covered with hair. It was attached rather broadly to the underlying structures by the connective tissue and skin. The mass contained a considerable amount of blood but no pigments or cysts were observed.

* Received for publication February 27, 1925.

Pathological histology. Sections were prepared from various portions of the submitted material and stained with hematoxylin and eosin.

The low-power view presented an atypical structure containing a number of irregularly shaped alveoli lined with cuboidal epithelium. For the most part of the cells lining the alveoli were in a single layer, although many alveoli showed an excess of these cells usually at one side, as though infiltrating into the adjacent stroma (see Fig. 1). Most of these alveoli were filled with a clear homogeneous substance that stained a bright pink with eosin, and which gave the characteristic orange-yellow reaction of epithelial hyalin, with Van Gieson's stain, although some of the material within the alveoli gave a light rose red reaction by the same method. In the areas between the alveoli the connective tissue was scant. Most of the space was occupied by what appeared to be epithelial cells morphologically similar to the cells lining the alveolar spaces. These cells were closely packed in small nestlike compartments, which arrangement was very apparent under the high power (see Fig. 2). Some fields contained large irregularly shaped areas, consisting of a hyaline-like substance which appeared vacuolated, and which stained a lavender color by Van Gieson's method. The nestlike groups of epithelial cells were few in these areas, and the number of cells making up the individual groups was a great deal less than in the other parts of the field and many showed changes suggestive of atrophy. Around many of the epithelial nests, and in the lumina of most of the alveoli were many vacuoles of variable size and shape (see Fig. 1). Red blood corpuscles were abundantly distributed in capillaries throughout the scant connective tissue of the stroma, and large blood vessels with well-defined walls were numerous. A few of the larger vessels showed thrombi formation apparently of some standing.

Viewed under high power the individual epithelial cells were spherical and oval in shape, with considerable variation as to size and staining capacity of the nuclei. Some, mostly of the small variety, staining with deep intensity so that chromatin granules were not discernible while others stained lightly except for the chromatin material which appeared as minute granules. The nucleus occupied most of the cell, the cytoplasm being so small in amount as to escape detection in most instances. Actual mitosis was not prevalent, al-

though there was enough evidence of this to indicate some aggressiveness on the part of the tumor cells (see Fig. 3).

Diagnosis. The epithelial character of the cells arranged in alveolar and nestlike formations, together with evidence of mitosis and infiltration would stamp this as an adenocarcinoma.

There is sufficient evidence, I think, to feel reasonably sure that the type cell in this formation had a common origin with the epithelial cells making up the parenchyma of the normal thyroid gland. The tendency toward alveolar arrangement and the presence of colloid material makes this obvious, even though the attempt at alveolar formation was not successful throughout. However when one considers the location of the tumor it is somewhat difficult to give an acceptable explanation of its position that is in keeping with current embryological teaching although its heterotopic origin is possible from some prenatal mishap, involving one of the lateral thyroids. From the standpoint of a metastatic tumor from the thyroid gland the task of explaining its presence would not be so difficult if it were possible to examine the gland, as it might present some interesting features bearing upon the possible origin of this tumor. Any change in the thyroid in this animal apparently was not obvious, otherwise the gland would have been removed at the time of slaughter.

While the bulk of the structure was decidedly infiltrative in character, yet the scarcity of mitotic figures together with the age of the animal and the apparent lack of metastatic foci would indicate a tumor of slight progressiveness.

Some of the microscopic features are difficult of interpretation, especially the hyaline-like areas which gave the atypical reactions to Van Gieson's stain. It is my opinion that the substance making up these areas and the hyaline contents of the alveoli had a common origin, and simply present different stages of the same material. Here we are confronted with the usual obstacles one encounters when attempting adequately to describe and interpret a hyaline change.

The vacuoles are possibly the spaces vacated by the removal of fat droplets since the tissue had been preserved in alcohol for some time. For this reason there was no opportunity of preparing sections for a specific fat stain.

DESCRIPTION OF PLATE LIV

FIG. 1. Adenocarcinoma resembling the thyroid gland. Low-power photomicrograph, showing irregular hyaline-like areas, alveoli, vacuoles, etc.

FIG. 2. Adenocarcinoma resembling the thyroid gland. High-power photomicrograph showing nestlike formations of tumor cells.

FIG. 3. Adenocarcinoma resembling the thyroid gland. High-power photomicrograph showing one cell undergoing mitotic division.

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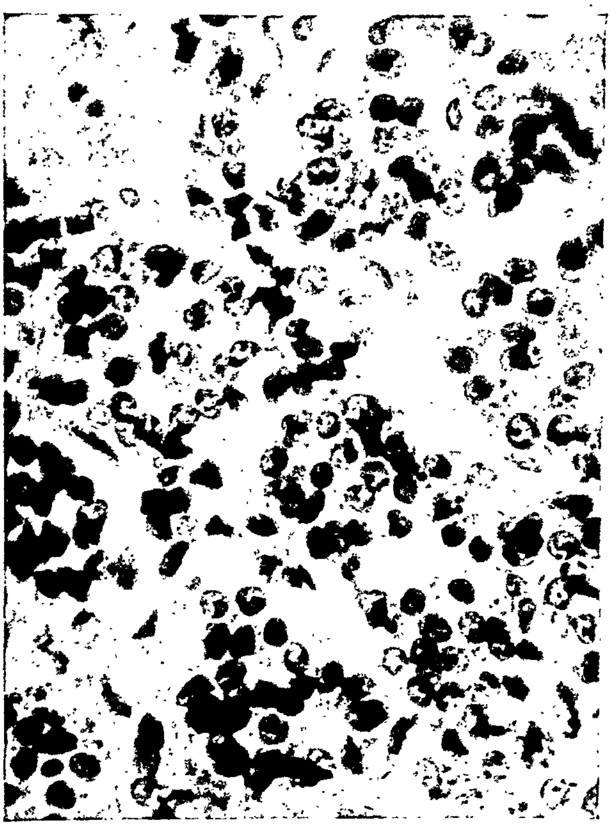

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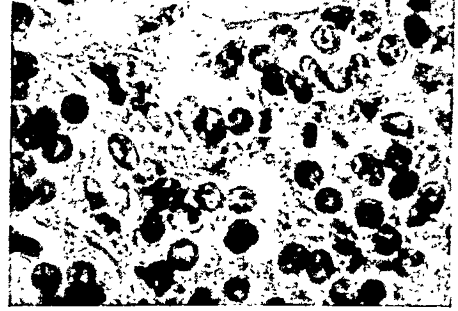

3

Feldman Adenocarcinoma Resembling Thyroid Gland

TISSUE CHANGES AFTER EXPERIMENTAL DEEP ROENTGEN IRRADIATION *

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It is a well-recognized fact that the influence of Roentgen irradiation upon the organism as a whole is just as important as its influence upon the pathological condition itself and may be more so. That fatal intoxication may be a sequel to intensive Roentgen therapy is a fact which is known from the altogether too rare publications of such misfortunes. Patients treated intensively with Roentgen rays very frequently react with general malaise, prostration, vomiting, diarrhea, loss of appetite, weak pulse and headache, as may be ascertained by anybody who has had the opportunity to observe even a limited number of such patients.

In the opinion of Miescher [1] the clinical incidence of radiation toxemia is about forty per cent of all cases treated, this condition being most marked when the radiation was over the epigastric area. Groedel and Lossen [2] met with radiation toxemia in twenty-five per cent of all cases when the head and neck were exposed, in thirty-three per cent in irradiation of the thorax and in fifty per cent in irradiation of the abdomen. This constitutional reaction does occur, though varying in degree, not only in deep Roentgen therapy but also in irradiations with the long-waved soft rays, a fact which has not been sufficiently stressed in the literature. Twenty years ago Seldin [3] tried to explain the cause of "Roentgenkater" by experiments on guinea pigs. A line of investigators followed him in an attempt to solve this difficult question. Some brilliant work was done recently by American and British investigators who contributed much to the pathological physiology of irradiation intoxication. However, this question is still far from a satisfactory solution.

What is the cause of irradiation toxemia? To discuss the literature of this question would mean that one would become lost in a maze of opinions. This may be illustrated by the mention of just a few of the multitude of theories and hypotheses: Gas poisoning

* Received for publication January 30, 1925.

through poor ventilation of the Roentgen room, induced high voltage of the patient's body, toxic nephritis with acute uremia, retention in the body of toxic products of nucleo-protein decomposition, anaphylaxis, psychical sickness. Lange[4] speaks of acidosis developed in the irradiated body as the cause of intoxication and advises prophylactic treatment with alkalies, while Myers [5] speaks of alkalosis in which alkali therapy would greatly aggravate the condition. As may be seen this question of the cause of irradiation toxemia is still a field of speculation where hypotheses greatly overwhelm proved facts. There are, however, features in the pathology of Roentgen intoxication which are of interest even if they alone are insufficient to explain the condition. These features are the morbid anatomical changes.

In 1921 Wetzel[6] in Europe and in 1924 Case and Warthin[7] in this country called attention to certain changes in the liver observed in patients who were treated with deep Roentgen therapy and died afterwards. In the pathological study of patients who have died after therapeutic irradiation the pathology of the condition for which radiation was done has to be considered. This fact makes the very limited human material available for pathological study of questionable importance. It is evident, therefore, that the pathology of Roentgen irradiation may be best studied by experimentation on animals.

Most experimental work in this line has been done since the invention of the Coolidge tube. From the published reports it would seem that most authors did not pay due attention to the question of the amount of X-ray energy given the experimental animal. The data usually given in the reports: the voltage, skin target distance, time of treatment, amperage and filter, are not sufficient for an exact measure of the amount of X-ray energy given. Insufficient attention has been paid to the readings of the electrostatic indicator, which vary with the tube used and vary also in the same tube at different times. There can also be no question that if a guinea pig is exposed to the same amount of X-ray energy as a dog, or a dog to the same amount as a human being the morbid changes produced will not be characteristic of the changes produced in patients by Roentgen therapy. Inattention to these facts may explain some of the discrepancies in the opinions of the investigators as to the results of the experiments.

In this work an attempt has been made to study the morbid changes in the liver, kidneys, spleen, and intestinal tract in experimental irradiation of rabbits. Two series of experiments were conducted. In one the rabbits were exposed to an X-ray energy, in proportion to their average weight, about twice as high as the energy to which patients are exposed in the usual intensive deep Roentgen therapy. The amount of X-ray energy and the dose given these rabbits can be calculated from the following data: 5 milliamper tube current, 200.000 volts (peak), filter 0.53 mm. Cu., skin target distance 70 cm., 1950 "e" units of X-ray energy per sq. cm. exposed, the time of exposure two hours. The whole body was exposed. In the second series the epigastric region of the rabbits was exposed; the time of exposure one hour. All the other conditions in reference to the X-ray energy and the dose given were the same as in the first series. Under these conditions the rabbits of the second series were given an irradiation, in proportion to their average weight, equal in intensity to that which is usually given to patients in deep Roentgen therapy. All rabbits died from the third to the ninth day after exposure. There was no appreciable difference in the changes in the organs studied between the two series.

The gross anatomical findings in the organs other than those described below were of no interest. The liver, the kidneys, the spleen and the intestinal tract were studied in gross and microscopically. They showed the following changes.

Liver. There were no appreciable gross changes in the liver except for scattered pinpoint- and pinhead-sized areas which could be perceived by means of a middle-power magnifying lens. These areas were distinctly different from the surrounding liver tissue. Their gross characteristic, however, did not permit any conclusions as to their nature. Microscopically one sees in the liver passive congestion which is especially prominent during the first few days after irradiation. Beneath the capsule of the liver there is marked dissociation of the liver cells with swelling of the cytoplasm and weak staining of the nuclei. The cytoplasm has a granular appearance. Deeper in the liver parenchyma are found scattered numerous small necrotic foci. Here may still be found liver rods, composed of cellular elements, which have undergone necrotic changes. These necrotic areas are surrounded at their periphery by liver cells still with staining nuclei but with broken-down cytoplasm, showing marked hy-

dropic changes. In the immediate vicinity of the necrotic foci congestion is very prominent. Here is seen some lymphocytic infiltration, especially in the older cases. Further to the periphery the liver cells are pyknotic, the nuclei stain deeper but seem of smaller size without the characteristic vesicular appearance. The Kupffer cells throughout the liver contain granular inclusions, consisting of chromatin and hemosiderin. The blood vessels in the necrotic areas are thrombosed; in some of them the organization of the thrombi is well advanced.

The last to undergo degeneration seem to be the bile ducts, so that in distinctly necrotic areas more or less normal bile ducts are still found. However, in the central portions of the necrotic foci the bile ducts are necrotic, traces of the columnar lining epithelium and accumulations of bile pigment scattered in the necrotic tissue being the only signs of former sites of bile ducts. On the whole a hyperplasia of the bile ducts is seen as may be judged from the character of their lining epithelium as well as from the rapidity with which they regenerate in the necrotic foci.

At a later date (five to eight days after exposure) one finds an interesting picture of repair in the injured liver. In the central portions of the necrotic foci are still present masses of cellular débris, which have not been as yet removed by autolysis, absorption and phagocytosis. The periphery of the necrotic areas begins to show all signs of repair. Connective tissue has spread from the periportal spaces far into the necrotic foci. Young fibroblasts with fusiform and vesicular nuclei have invaded this area. In the peripheral portions, where repair began at a somewhat earlier date, may be found groups of liver cells invading the repaired area. These liver cells do not show the degenerative changes found in the old liver tissue surrounding the necrotic foci; they seem to be new-formed elements. In the area of repair may be seen old bile ducts which did not succumb at the time when the liver cells became necrotic; but also numerous new-formed bile ducts are found. Some of the latter show small lumina, lined with more or less cuboidal epithelium. The majority are, however, in the very young stage when no lumina are seen as yet. They are formed by long groups of cells with relatively large nuclei and small amount of cytoplasm. On a cross section they appear as giant cells with nuclei placed at or about the periphery, with the center (lumen) taken up by the syncytial cytoplasm which

does not show as yet clear cell borders between the individual cellular elements. Special staining shows the connective tissue wall surrounding these young ducts. Such "giant cells" could never be found in lumina of larger bile ducts. Considerable thought and careful examination was given to this question, because of a recent paper by Case and Warthin [7] in which the authors speak of giant cells obliterating the bile ducts in the livers of patients who died after intensive deep Roentgen therapy for abdominal malignancy.

Kidneys. Several theories attempting to explain the irradiation toxemia are based upon the temporary depression of the function of the kidneys. The recent hypothesis of Anderson and Kohlmann [8] that Roentgen sickness is due to an acute uremia is the most well-developed of these theories. However, the question of the changes in the kidneys following intensive irradiation is still an open one. In these experiments the kidneys showed at all times after irradiation an extensive focal parenchymatous nephritis. Congestion and cloudy swelling of the cortex were the main characteristic changes in these foci. The desquamated lining cells of the tubuli frequently formed granular casts. The glomerular tufts showed the same degenerative changes to some slighter degree. Free blood was found frequently within Bowman's capsule. The medullary portion of the kidneys did not seem to be affected by the process.

Spleen. The spleen is markedly changed grossly. The decrease in size is astonishing. The capsule is redundant and laid in folds. As a matter of fact in two cases it was difficult to find the spleen at autopsy. In each rabbit only a small piece of dark gray tissue was present at the splenic site.

The changes are even more marked on microscopic examination. Only very small remnants of the Malpighian bodies are left. The main bulk of the remaining splenic tissue is made up of the splenic pulp. Moreover there is a conspicuous scarcity of lymphoid cells in the pulp. Very few lymphoid cells can be found in the remaining small Malpighian corpuscles. The leading elements of the small corpuscles now are the long spindle-shaped cells with bulging nuclei. In contrast to the liver and kidneys the spleen is very anemic. The central arteries and cavernous veins seem to be collapsed, especially the latter. At the same time there are seen in the reticular meshes numerous broken-down red blood cells.

The large ameboid phagocytic cells of the splenic pulp contain abundant granular inclusions consisting in the main part of hemosiderin. Along with hemosiderin these inclusions contain cellular débris of disintegrated lymphocytes. As may be seen here, as in the liver, the reticulo-endothelial apparatus participates in the phagocytosis of the remnants of the disintegrated cellular elements and especially of the end products of the disintegrated red blood cells.

Intestinal tract. The changes in the intestinal tract after intensive irradiation have long been known from experimental as well as from clinical study. The intestinal tract permits the best systematic study of its anatomical changes after irradiation. One finds there different degrees of involvement from the most inconspicuous desquamation of the epithelium lining the tips of the glands of the intestinal mucosa to the most serious deep ulcerations of the intestinal wall with impending perforation of the gut. In twelve hours after epigastric irradiation may already be found a desquamation of the mucosa epithelium, no hemorrhage or cellular infiltration being present as yet. Soon changes in the blood vessels appear. The endothelium lining them swells, becomes granular and desquamates. Thrombosis of the vessel follows. The extensive thrombosis leads to necrosis, to ulceration of the intestinal mucosa and submucosa. A hemorrhage in the immediate vicinity of the necrotic area leads to a picture which can be well seen grossly as a dark red ulcer of the mucosa. Necrosis spreads deeper into the wall and in one case was found a perforation of the intestinal wall with a subsequent general peritonitis. A marked lymphocytic infiltration was found to be constantly present in the pathological picture. However, the lymphocytic infiltration is of a far less degree than one would expect in such a process as an ulceration of the intestinal mucosa. In contrast to the liver one does not see any signs of repair in the intestinal mucosa. In all cases the ulcers progressed until the death of the animal took place. In one of the rabbits ulceration occurred also in the vermiform appendix of the cecum, thus producing an acute gangrenous appendicitis which led to perforative peritonitis and death.

Discussion

Such are the morbid changes which took place in the organs of the experimental animals. An analysis of these results shows certain features which are common to all of the organs studied. One of these

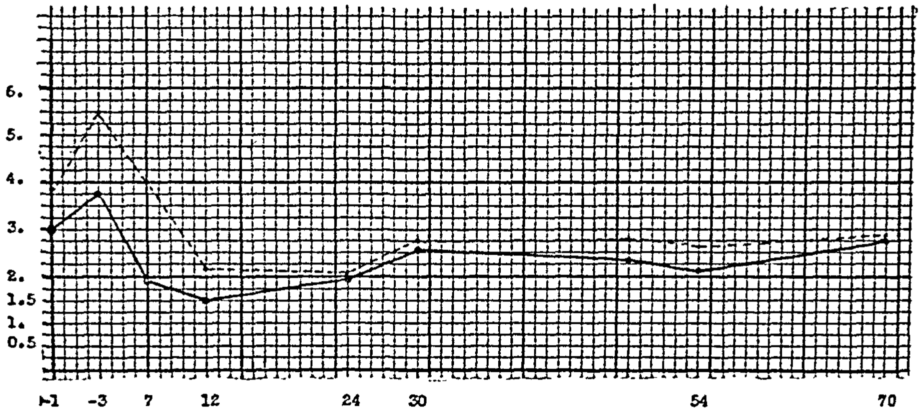
is the changes in the blood vessel walls, changes which lead to extensive thrombosis. The destruction of the erythrocytes is another characteristic feature of the changes induced by intensive Roentgen irradiation. But the most conspicuous of these features is the rarity and scarcity of lymphocytic infiltration accompanying the inflammatory and necrotic changes of the tissues, and the enormous destruction of lymphocytes in the spleen.

The destruction of lymphocytes, the disappearance of the Malpighian follicles, and the overgrowth of the reticular elements of the splenic pulp suggest the possibility of an influence of Roentgen irradiation upon the coagulability of blood. The two leading theories regarding coagulation of blood are based upon the hypothesis that the spleen plays a specific rôle in the determination of this process. According to one the reticular cells of the splenic pulp are specific for blood coagulation. Stimulation or increase in number of these elements will therefore lead to an increase in coagulability of the blood. The other theory, supported by the majority of investigators, claims that a destruction of the splenic follicles leads to a liberation of thrombokinetic or thromboplastic substances. The changes in the spleen of the rabbits are of such a nature that accepting either one of these two theories an increase in coagulability of the blood after irradiation could be expected.

To study the influence of irradiation upon coagulability of the blood two series of experiments on rabbits were conducted. The epigastric region in normal rabbits was exposed to X-rays. The dose of X-ray given was, in proportion to the average weight of the rabbits, equal to about one fifth of an intensive therapeutic dose for a human being. The coagulability of blood was studied after the method of Brodde-Rossell and by the capillary method. The curve given in text figure 1 illustrates the findings. It may be seen that there is a definite increase in coagulability of the blood (a decrease of time in which coagulation takes place) after irradiation. The highest coagulability was observed at about twelve hours after the exposure. Experiments on rabbits in which various other regions were exposed gave similar results, though the increase in coagulability of the blood was less marked.

A further study of the literature showed that in 1920 Stephen [9] discovered that irradiation by Roentgen rays increases the coagulability of blood. He then suggested the use of irradiation in surgery

when an increase in coagulability is necessary for a surgical interference. This suggestion has not gained ground, apparently because of the fact that the influence of irradiation upon coagulability was not understood biologically. Though the question of the influence



TEXT FIG. 1. Curve illustrating the influence of Roentgen irradiation on the coagulability of blood. The continuous line is the result of the Brodde-Rossell method. The broken line of the capillary method.

of irradiation upon coagulation of blood has not been wholly answered by the described results of these experiments, it would seem, however, that the pathological changes in the spleen after irradiation suggest the biological explanation of this influence.

CONCLUSIONS

Experimental intensive deep Roentgen irradiation produces definite anatomical changes in the liver, the kidneys, the spleen and the intestines, which play a certain rôle in the etiology of radiation toxemia.

The main features of these histopathologic changes are:

1. A destruction of lymphocytes, which is especially prominent in the spleen.

2. A disintegration of erythrocytes in all the organs studied with abundant hemosiderin formation.

3. Degenerative vascular changes with subsequent thrombosis of the vessels.

The destruction of lymphocytes and the disappearance of Malpighian follicles of the spleen with the overgrowth of the reticular elements of the splenic pulp are the biological basis for the increase of the coagulability of blood after irradiation.

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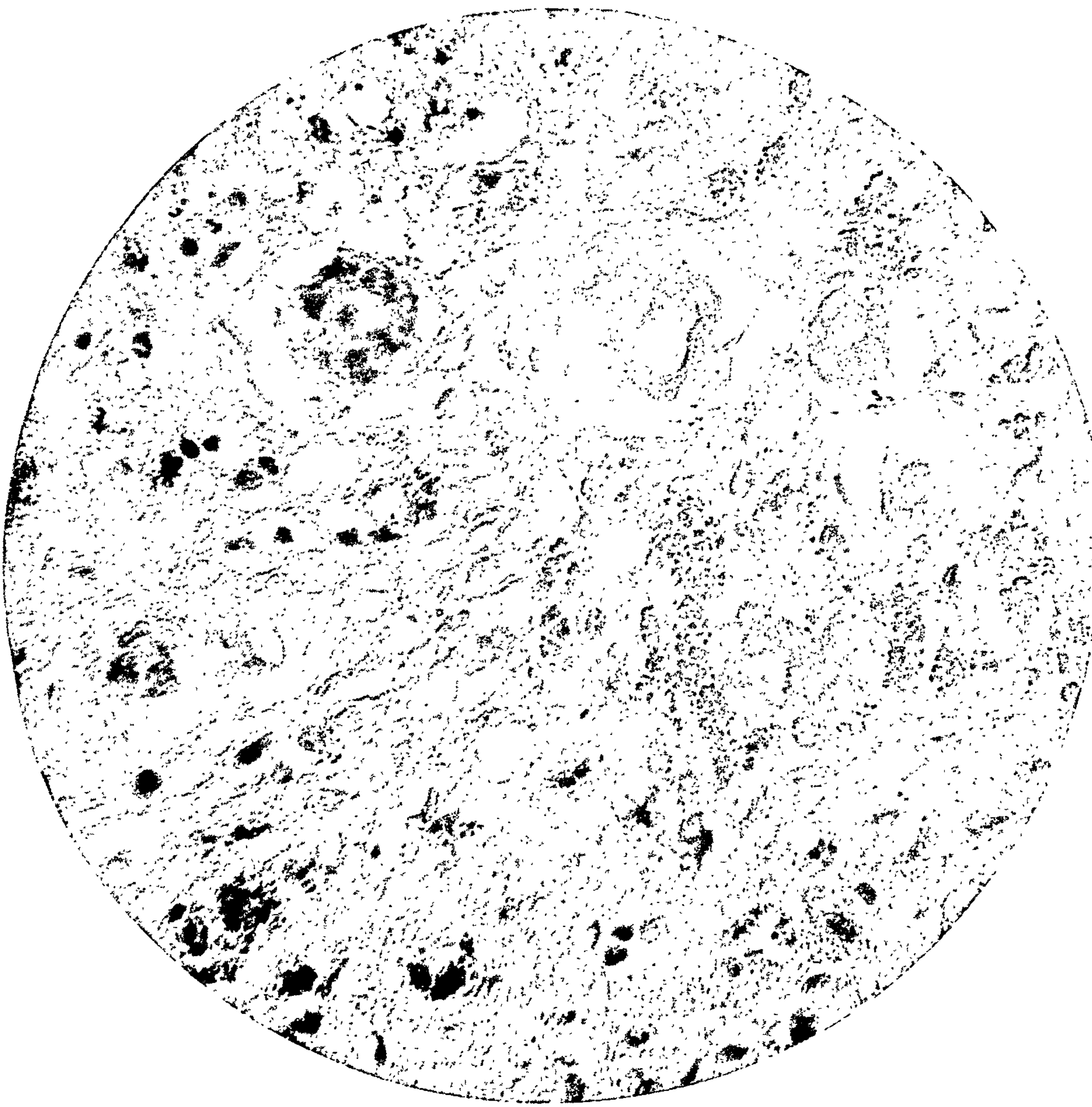
DESCRIPTION OF PLATE LV

FIG. 1. Regeneration of bile ducts in a necrotic focus of the liver where repair is well advanced. The youngest of the bile ducts do not show as yet any lumina, and on cross section they simulate giant cells.



Kolodny

Tissue Changes after Irradiation

STUDIES ON MITOCHONDRIA

I. THE CHANGES OCCURRING DURING EXPERIMENTAL THYROID HYPERPLASIA AND ITS INVOLUTION WITH IODINE*

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According to Grynfeltt[1] the mitochondria in the thyroid were first studied specifically by O. Schultze[2] in the rat. Later they were described by Mawas[3] in the rabbit, and by Grynfeltt[1] in the horse, ox, sheep, and kitten. In all the animals studied the findings were essentially similar. In the "normal" glands they found variable numbers of granules, rods and filaments, the latter oriented parallel with the long axis of the cell. They also noted that the mitochondria were usually more numerous near the lumen surface of the cell. Attempts were made to associate the variations in the number and shape of the mitochondria with alterations in the specialized activities of the cells, as for example the production of secretion, participation in colloid formation, and other possible physiological functions.

In the light of the recent literature, it appears very unlikely that mitochondria are concerned with the specific activities of cells, but they may, perhaps, participate in the basic cellular activities such as metabolism and respiration.

It is safe to assume that "even if the mitochondria are as inert as iron filings their presence in such variable amounts must surely exert some influence upon the activity of the cells containing them, and we have good reason to think they are not chemically inert substances" (Cowdry,[4] p. 82). Moreover, there is a growing conviction that "variations in the shape and size of mitochondria constitute by far the most delicate criterion of cell injury known to us. Mitochondria react long before the nucleus, and their morphology is the first thing to change, though it is soon followed by alterations in distribution and in amount" (Cowdry,[4] p. 71).

Our findings on the variations in number, form, and distribution of mitochondria in the thyroid cell may modify some of the current

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opinions regarding the anatomical evidences of thyroid "activity" and have some bearing on the question of morphological indicators of cellular activities in general.

In 1916 Goetsch [5] reported that in the thyroid adenomata from cases diagnosed "toxic adenoma" the mitochondria were increased in number over the normal, although with ordinary methods of fixation and staining the sections failed to reveal any evidence of over-activity, i. e., hyperplasia. Upon the assumption that this increase is associated with an increased activity (secretion) of the cells he believed that there was a correlation of the clinical manifestations of thyroid hyperfunction with the anatomical changes in the gland — when the mitochondria were used as the criterion of activity. According to Cowdry [4] (p. 136), "the correlation of the clinical manifestations of hyperthyroidism and the mitochondrial picture is such that Goetsch is justified in saying that the two are definitely related. His conclusions are based upon the study of approximately 125 cases of thyroid disease in the human." Concerning the significance of Goetsch's discovery, Cowdry [4] states (p. 44), "It has come to be an embarrassing question to ask one to pick out from a number of thyroids, on the basis of the histological appearance, the one which was associated with the clinical symptoms of hyperthyroidism. The height of the epithelium, the appearance of the nuclei, and the amount of colloid are, at best, but poor aids in the dilemma. Now Goetsch has discovered that, in the cases which he has observed, the mitochondria are enormously increased in number where there are symptoms of hyperthyroidism. In other words, he has succeeded in correlating the perplexing clinical symptoms in the little-known condition of exophthalmic goiter, or Basedow's disease, with a definite anatomical change in the gland itself (i. e., in the mitochondria)."

Key [6] and Bolt [7] have reported similar increases in the number of mitochondria in pathological thyroids. The latter emphasized that this finding in the "colloid goiters" established the anatomical basis for the clinical symptoms of hyperactivity of the gland in the absence of the commonly sought for hyperplasia.

Working with routine histological methods, Marine showed that the anatomical variations of the thyroid were due to variations in the percentage-content of iodine in the gland irrespective of the clinical syndromes with which the variations were associated. All attempts to reconcile the anatomical variations with the functional

disturbances based upon clinical conceptions of its activity have failed. This failure, no doubt, is largely due to our incomplete knowledge of the physiology of the gland against which we are attempting to titrate its anatomical pictures. The definite association of clinical symptoms with specific mitochondrial changes by Goetsch appeared to confirm the clinical conceptions of the functional activity of the thyroid upon an anatomical basis — not evident in routine histological sections, but hidden in the finer cytological changes in the cells.

What would appear to be the best confirmation of Goetsch's work is found in the inability of Nicholson [8] to produce by various experimental procedures (except in compensatory hypertrophy) an increase in the mitochondria in the thyroids of animals, even though he resorted to manipulations calculated to alter the respiratory and metabolic activities of the thyroid cells. Many of his manipulations resulted in a decrease, and to quote him (p. 73), "In no one case has it yet been possible to bring about experimentally an increase in the length of the mitochondrial filaments — that is to say, to produce a change such as Goetsch [5] discovered in exophthalmic goiters." (We could find no mention of the increase in length of the mitochondrial filaments in the reference [5] cited by Nicholson or in any of the publications by Goetsch.)

The results of Nicholson's experiments implied that mitochondria were perhaps not so sensitive to variations in the metabolic activities of cells as previous work had suggested. Goetsch's findings implied that alterations in the mitochondrial constituents of the thyroid cells were indicative of variations in the functional activities which were associated with clinical syndromes.

The exact significance of mitochondrial variations in cells in general, and in the thyroid in particular is unknown. It occurred to us that we might arrive at a better understanding of these by recording the variations in the mitochondria, if any, which take place in the thyroid cell, when these changes could be titrated against a well-known and controllable activity of the gland. We had in mind the response of the thyroid cells to disturbances in the iodine content of the gland. This report deals with the alterations in the number, distribution, and morphology of the mitochondria of the thyroid cell during the passage of the gland from the involuted, or "normal" state, to the hyperplastic state, and vice versa.

Methods and Materials

Though the underlying mechanism and significance of the hyperplasia are not yet clearly established, its experimental production in animals by partial removal was demonstrated by Halsted [10] and by high fat feeding by McCarrison.[11] Both have been confirmed by others. As in all other physiological hyperplasias of the thyroid, involution is brought about by the administration of iodine. We have studied the mitochondrial variations during the developmental stages of hyperplasia induced in the thyroids of cats, rats, rabbits, guinea pigs, pigeons and chickens by high fat diets, and during compensatory hypertrophy in the guinea pig, as well as those occurring during the involutionary response produced by iodine. In this paper we wish to report particularly our observations on the rat and guinea pig, although they were the same in all the animals studied.

Two rats were killed at the beginning of the experiment as controls and the remainder of a series of twenty were fed 3 c.c. of cottonseed oil daily in addition to their standard rations. By killing pairs at weekly intervals we obtained a series of glands showing the progressively increasing degrees of hyperplasia during nine weeks of the fat feeding. The changes occurring during the involution phase were studied in guinea pigs by the administration of iodine after the hyperplasia was well established.

The thyroid specimens were fixed in Regaud's solution and stained according to a slightly modified method as given by Cowdry [4] (p. 60) with acid fuchsin methyl green.

Observations

In the "normal," low cuboidal cell the mitochondria are few in number, scattered diffusely, though slightly more numerous at the lumen pole of the cell, and are chiefly granular with occasional rods and filaments. Concomitant with the appearance of the histological evidences of hyperplasia, namely, an increased vascularity and diminution in the colloid content of the gland, and a hypertrophy of the epithelial cells there occur characteristic alterations in the number, distribution and morphology of the mitochondria in the thyroid cell. In the hypertrophic cell, the number is increased * so that the cyto-

* We are inclined to believe that the increase in the mitochondrial substance is absolute, and independent of the increase in the volume of the cytoplasm. It would be of interest to attempt a study of the mitochondrio-cytoplasmic relationship in the

plasm is actually filled with the red staining elements; there are huge condensations at the lumen or apical pole; and in form, they are almost purely filamentous. The shorter types are the transversely cut or fragmented forms of filaments. The filaments are oriented perpendicularly to the basement surface of the cell, fairly straight in the shorter forms, but distinctly wavy when they extend from the base to the free surface or are lost in the apical masses. The accumulations at the apical poles are often so dense that the individual forms are barely discernible.

When on the administration of iodine the gland begins to involute and the cells to flatten, the mitochondria begin to diminish in number, the apical clumps decrease, and granular and rodlike forms appear in the cells. In the flattened cells of the completely involuted gland only an occasional granule is visible.

Discussion

In the hyperplastic thyroid we have found an *increase in the number of the filamentous type of mitochondria throughout the entire gland.* If the increase occurred only in the number of mitochondria this might be attributable to the fact that different portions of the gland were compared, or that different sections were unknowingly subjected to slightly varied conditions during fixation or staining, or to the fragmentation of the larger forms. It is well known that the mitochondrial elements are very susceptible to injury by technical manipulations. The conflicting opinions regarding their number and morphology in the same tissues, under apparently the same conditions, may in a large measure be accounted for by the differences in the technique used by the various workers. We have employed several different methods of fixation and staining but in our hands the Regaud fixation and a slightly modified Cowdry method of staining with fuchsin and methyl green yielded the most satisfactory specimens. We can confirm Cowdry's [4] statement (p. 64): "When fixatives do alter mitochondria, they alter them in a definite way. Filamentous mitochondria break up into granules and spherules. Filamentous mitochondria are never formed by the fixative from

thyroid cell similar to that made by Thurlow [9] in nerve cells. In the thyroid cell, where the variations in the cytoplasmic volume is experimentally controllable the results may be more conclusive and the significance of the disturbances, if any, may be more easily determined.

granules." The preservation of the filamentous structures intact is the best check on our technical methods.

Mitochondria and Functional Activity of the Thyroid. The thyroid cell is very labile. In any large series of animals kept under apparently similar conditions the thyroid mitochondria show marked individual variations. It is therefore obvious that such animals cannot be used as "normals" as many students of this subject have done. We involute the thyroid cells to uniformity by the administration of iodine before beginning our studies.

The inability of Nicholson to produce an increased number and elongation of the mitochondria may be due to the fact that he was unable to produce hypertrophic cells. In the hyperplasia which he obtained following partial removal, his failure to observe the increase in the filamentous forms may be due to some unrecognized error in technique. For, in a small series of guinea pigs in which we studied the changes during compensatory hypertrophy our specimens showed the same qualitative and quantitative variations, according to the degree of hypertrophy, as was described in the hyperplasias produced by the fat feeding. It is worthy of note, however, that Nicholson found that the administration of iodine or thyroid substance to his "normal" animals produced a "fragmentation" of the filaments, and a diminution in the number of the mitochondria.

The findings in the thyroid of humans are to be interpreted very cautiously for here we are confronted with the so-called "adenomata," the formation and significance of which do not concern us here. When once formed, however, Marine[12] has shown that their response to the administration of iodine is abnormal. While the remainder of the gland may be involuted the adenomatous tissue may persist in its hyperplastic state, or under conditions bringing about hyperplasia of the normal portions of the gland, the adenomatous structures remain unchanged. The significance of this fact is unknown, but it enables us to attempt an explanation of the findings in the human material. The presence of the large numbers of mitochondria in the non-hyperplastic adenomatous tissue observed by Goetsch and Bolt may be accounted for on the assumption that the cells had either just begun to hypertrophy or that the flattening had just occurred. and that the mitochondrial delay was an indication of the tardy response of the cell to influences which had produced the involution. If it could be demonstrated that the response of the

mitochondria becomes evident before or persists longer than the changes which occur in the remainder of the cell, the possibility that these adenomatous structures are indicative of some form of activity not shared with the remainder of the gland might be granted. It is not likely, however, that this difference would be detectable by clinical symptoms. For, with the development of the hyperplasia and its involution by iodine, during which the mitochondrial variations take place, there were no clinical evidences of disturbances in the functional activity of the thyroid that we were able to recognize. We may add also, that we have seen specimens of glands from cases of exophthalmic goiter showing the involuting effects of the iodine which had been administered therapeutically, and in spite of the marked reduction in the mitochondrial contents of the cells there was no parallel decrease in the severity of the clinical symptoms.

The final proof that an increase in the number of mitochondria in the thyroid is not associated with an increased functional activity based upon clinical symptoms will be given when it is demonstrated that in cretinism and myxedema, in man and in animals, the mitochondria in the hypertrophic thyroid cells are similar to those described in the hypertrophic thyroid cells of our experimental animals.

Mitochondria and Cellular Activities. The true significance of the mitochondrial variations in the thyroid cell may remain unknown until the relationship between the morphology of the thyroid and its iodine metabolism becomes established. Nevertheless, it is apparent that a single though definite "activity" of a highly specialized cell has been correlated with definite and striking alterations in its mitochondrial contents. The specific affinity of the thyroid cell for iodine which enables one to produce experimentally alterations in its mitochondrial constituents may offer a suitable means of studying certain problems.

It is beyond the scope of this communication to enter upon a discussion of the various theories regarding the relationship between the mitochondria and cellular activities in general. They have been associated with every conceivable biological activity, from forming the cytoplasmic basis of organic inheritance to participation in histogenesis and in the specialized activities of highly differentiated cells — such as contraction, secretion and conduction. Goetsch looked upon them as indicators of clinical syndromes. Others have regarded

them as bacterial symbiotes of cells. The conflicting evidence for and against these views is outlined in the extensive monograph by Cowdry.[4] For the present we may state that most of the evidence was deduced from the alterations in the number, form, and distribution of these elements under the conditions investigated.

It is quite possible that the conflicting results under the same conditions are due to unrecognized errors or variations in the technique employed by the different observers. We are now enabled to study accurately some of the factors concerned with the preservation and disintegration of the filamentous forms in fixed tissues by checking the technical variants against the mitochondrial pictures in the hypertrophic thyroid cell, in which we know that the mitochondria should appear as large numbers of the filamentous type.

Regardless of what the significance may be the fact is that in the thyroid cell the increase in the volume of the cytoplasm and nucleus is accompanied by an increase in the number and length of the mitochondria. As yet we have been unable to determine whether the changes take place first in the nuclear, cytoplasmic or mitochondrial constituents, and whether in the latter the number, morphology or distribution is first to be altered.

The ability experimentally to alter the distribution of the mitochondria may offer a suitable method for studying their relationship to cell polarity and the secretion and absorption of the colloid in the gland. It is evident that the largest amount of mitochondria is present in the thyroid cells when the colloid content of the gland is least. This fact may have some bearing on the chemical processes in the gland and on the question of the chemical constituents of mitochondria.

Summary and Conclusions

The number, morphology and distribution of the mitochondria in the thyroid cell vary directly with the degree of active hyperplasia or involution. During the developmental stages of active hyperplasia the mitochondria increase in number, become filamentous and concentrate at the lumen pole of the cell. During involution the mitochondria decrease in number, become shorter and more scattered.

The cycle of histological changes in the thyroid has been shown by Marine to be dependent upon variations in the percentage-iodine

content. Our studies on mitochondria indicate that the variations in these elements also depend upon the same factor, and therefore cannot be correlated with existing clinical classifications of thyroid diseases.

The relationship between variations in the mitochondrial constituents and the functional activities of cells is unknown. The specific affinity of the thyroid cell for iodine may offer a suitable method for inducing controllable mitochondrial alterations by which future studies may lead to a better understanding of the significance of such variations, and possibly the relationship between mitochondria and cellular activities.

The writer wishes to acknowledge his indebtedness to Dr. David Marine for his valuable assistance throughout the progress of the work.

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THE ORIGIN OF THE MONONUCLEAR PHAGOCYTES OF PERITONEAL EXUDATES *

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That the chief source of the granular leucocytes is the bone marrow has been definitely established. Also lymphocytes of the usual type without doubt are derived from lymph glands and elsewhere from lymphoid tissue. Much investigation of the group of mononuclear phagocytes found in the blood and extravascularly has not so far resulted in a satisfactory explanation of the origin and relationship of cells of this type. Two prominent views of the origin of such phagocytes are those of Maximow [1] who holds that they are derived from a mesenchymal cell, the "hemocytoblast," that has the ability to produce all blood and wandering cells, and of Mallory [2] who traces their origin to vascular endothelium. These two views in regard to the origin of the wandering phagocytes are referred to especially because they are conclusions arrived at in two essentially different ways: Maximow's observations were made on embryonic tissue and the tissue of adult lower animals in cultures and in fixed and stained specimens; Mallory's, on the behavior of human tissue subjected to various pathological influences.

Sabin [3] has supported the endothelial origin of the phagocytes but more recently Cunningham, Sabin, and Doan [4] conclude that these cells are derived in part from the reticulo-endothelium, in part from a specific mesenchymal cell which they found to be present in the splenic pulp. The author [5] investigating the mononuclear phagocytes of the blood devised methods for using carbon in suspension to mark the vascular endothelium and found that carbon-laden endothelial cells may undergo mitosis to become free in the blood stream. Foot [6] in a series of seven articles has described the behavior of cells of endothelial origin to carbon, colloidal dyes, and tubercle bacilli and has placed emphasis on the importance of ingested carbon in the identification of the phagocytes.

* Received for publication March 16, 1925.

Second only to the cells of the blood the free cells of the serous cavities, especially of the peritoneal cavity, have received the attention of experimental investigators. The author first became interested in the determination of the reaction to benzidin of the peritoneal cavity cells and later undertook an examination of them by the reaction to carbon in suspension, and by the methods of vital and supravital staining. Cunningham [7,8] in two papers has defined the characteristic features of the mesothelial serosal cells of the peritoneum. Sabin, Doan, and Cunningham,[9] examining peritoneal exudate cells by the method of supravital staining, found the phagocytic ones to consist of two distinct types. In their "macrophage" type the neutral red of the stain was taken up by the cells as large irregular granules scattered about in the cytoplasm, while in the second or "monocyte" type, which in the peripheral blood was identified as the "transitional" leucocyte, the neutral red was seen as granules arranged in the form of rosettes. The "rosette" type of cell was found in the spleen in large numbers and on this basis it was regarded by Cunningham, Sabin, and Doan [4] as arising from a "stem-cell of mesenchymal origin which is resident in the pulp." This view of the duality of the mononuclear phagocytes is at variance with the one previously expressed by Sabin [3] in which all of these cells were traced to the reticulo-endothelium.

The peritoneal exudates investigated by the author were produced by injections of India ink, trypan blue solutions, and whole rabbit's blood into the peritoneal cavities of normal rabbits, splenectomized rabbits, and rabbits receiving intravenous injections of India ink. In a routine examination of these exudates by the benzidin peroxydase method of the author [10] it was found that none of the mononuclear phagocytes reacted, the reaction being given only by the leucocytes with neutrophilic and eosinophilic granules. It may be remarked that benzidin-reacting mononuclear phagocytes are not found in the peripheral blood of the normal rabbit although they are quite abundant in peripheral human blood.[11]

Exudate by injection of trypan blue. This dye in amounts of 20 c.c. of a 2 per cent solution was injected into the peritoneal cavity of rabbits first for the purpose of producing an exudation of cells, and second for the vital staining of the tissues of the animals. After

intervals of about ten days, second and third injections were given. Exudate for examination was obtained by means of a syringe and fine needle, and examined at once with oil immersion lens. On the first day after first injections, polymorphonuclear leucocytes were quite numerous, but at the end of forty-eight hours the mononucleated cells predominated and of these an occasional one contained coarse blue granules. After second injections, the number of cells with coarse dye granules became greater. Preparations of exudate were examined on unfilmed and filmed slides. The dye films were made on slides by the method for supravital staining of Pappenheim [12] and Simpson.[13] This method was later made use of by Sabin, Doan, and Cunningham.[9]

A very small droplet of the exudate is pressed out from the syringe needle upon a cover-glass rimmed with vaseline and at once inverted and pressed out upon a slide covered with the dye film. The film of dye on the slide is made from a fresh mixture of equal parts of 95 per cent alcohol one-half saturated with neutral red (Gruebler) and of 95 per cent alcohol one-sixth saturated with Janus green (N. A. C. Co.). Several preparations should be made on slides with films of varying thickness for the reason that exudates with many blood corpuscles absorb more of the dyes. The lymphocytes and polymorphonuclear leucocytes are readily distinguished in the supravitally stained preparations. Both are small and although the latter have neutral red granules, the nuclear masses with granulation between them can be seen. The large trypan blue granules of the vitally stained mononuclear cells are soon stained with the red and assume a deep purplish tinge. In the vitally stained rabbits, omission of the Janus green gives better preparations. A majority of the mononuclear cells present contain numerous neutral red granules in the cytoplasm without any definite arrangement. There are occasional cells with the neutral red granules massed in a focal area of cytoplasm in the form of a rosette. The last two types were first described by Sabin, Doan, and Cunningham [4, 9] and are discussed at length in connection with the exudates obtained by the introduction of whole blood. For convenience of description the trypan blue cell may be spoken of as the "hyaline" type, the one with diffuse neutral red granules as the "non-rosette" type, while the third variety has been called the "rosette" type.

Exudate by injection of India ink. The India ink (Higgins') is injected intraperitoneally in amounts of 5 c.c. On the first day polymorphonuclear cells are numerous but after forty-eight hours mononucleated cells predominate. Examination by supravital staining shows a few "rosette" cells which usually contain one or more carbon particles. The "non-rosette" type well-filled with scattered neutral red granules predominates. The cells of this type have a few carbon granules. The "hyaline" type cells are also quite numerous. These cells have much the greatest capacity for phagocytosis of carbon with some cells containing a half-dozen granules and others fully packed with the carbon. In general this type heavily loaded with carbon tends to stain relatively little with the neutral red but after contact with the dye for a half hour a considerable number of irregular neutral red granules appear in the cytoplasm. Mitoses in both the "hyaline" and the "rosette" types have been seen in preparations made at the end of forty-eight hours.

Exudate by injection of whole rabbit's blood. This is the method of producing peritoneal exudate employed by Sabin, Doan, and Cunningham,[9] and has the advantage that it causes a marked exudation of the "rosette" type of cell. Ten to 20 c.c. of whole rabbit's blood are injected daily into the peritoneal cavity of a rabbit. By drawing 2 per cent citrate into a sterile syringe and forcing it out preliminary to the heart puncture a somewhat longer interval for the puncture and injection may be obtained. Many observations have been made on eight normal rabbits and two splenectomized ones. So far as concerns the mononucleated cells reacting strongly to the neutral red, namely the "rosette" and the "non-rosette" types, they were found to correspond in most particulars to the description of them originally given by Sabin, Doan, and Cunningham.[9]

In exudates containing large numbers of the "rosette" type of cells it has been constantly observed that there are many with neutral red granules massed in some portion of the cell but lacking the typical rosette arrangement. These forms, which morphologically appear to be transitions between the two types, lead one to think that the "non-rosette" may be an older, more differentiated form of the "rosette" cell. There is also a correspondence of other characters discussed in connection with the intravenous injection of carbon and the incubation in vitro with carbon suspensions. In the

lymph node where the "rosette" cell develops from a cell with only a minute granule of the neutral red there is evidence of an increased ability of the older cells to take up the dye. Sabin, Doan, and Cunningham, as previously stated, considered the "rosette" and the "non-rosette" type to be transitional blood leucocyte and macrophage respectively. The third or "hyaline" type of cell was not described by them.

Since the three types of phagocytes are constantly present in the exudate after a few intraperitoneal injections of whole blood, a single description will serve to illustrate their character. Many observations of peritoneal exudates by supravital staining were made on two splenectomized rabbits. On September 25, October 2, and October 6, 1924, one of these animals received 3 c.c. of India ink (Higgins' intravenously in order to mark the phagocytic endothelium. On October 7, it was injected with 15 c.c. of whole rabbit's blood into the peritoneal cavity and 3 c.c. of India ink into the ear vein. On October 8, the cells of the peritoneal exudate were chiefly polynuclears (10 per cent containing carbon) but there were a few "non-rosette" mononuclears (20 per cent with carbon). No "rosette" and no "hyaline" cells were identified; injected with 20 c.c. of whole rabbit's blood. On October 9, less than one-half were polynuclear leucocytes (carbon in 5 per cent). Of the large mononuclear cells the majority were of the "rosette" type described by Sabin, Doan, and Cunningham.[9] Next in number were the "non-rosette" cells with numerous scattered neutral red granules. The latter were much more phagocytic for red corpuscles and polynuclear leucocytes. Less than one in ten of the two neutral red types contained carbon. Carbon particles were somewhat less readily found in the "rosette" cells. As is usually the case when these two types are abundant there are transition forms with the neutral red granules massed at one point or another in the cytoplasm but not in the form of a typical "rosette." There is an occasional "hyaline" type of cell and almost all of these had phagocytized a lymphocyte or carbon granules or both. Injected with 10 c.c. of whole rabbit's blood. On October 10, the number of polynuclears had diminished and the "hyaline" cells increased in number. Almost all of the "hyaline" cells contained carbon and one with six large granules was seen. Splenectomy under ether anesthesia. On October 13, injected with 15 c.c. of whole blood

into the peritoneal cavity and 3 c.c. of ink intravenously. On October 14, few polynuclears. Of the mononuclears, three-fourths were of the "rosette" type and about one in twenty contained carbon. The "hyaline" cells were as numerous as the "non-rosette" type. On October 20, intraperitoneally 15 c.c. of whole blood and intravenously 3 c.c. of ink. On October 21, exudate unchanged. On October 30, intraperitoneally, 15 c.c. of whole blood and intravenously 3 c.c. of ink. On October 31, little change in exudate. On November 3, intraperitoneally 10 c.c. of whole rabbit's blood and no injection of ink. On November 4, at least 3 "rosettes" to 1 "non-rosette." There was less than 1 per cent of these two types with carbon. As usual there was less phagocytosis by the "rosette" type.

The "hyaline" type of cell was conspicuous on November 4 and at least one-fifth of all the mononuclear cells were of this type. Although the size of these cells was variable the largest ones present in the exudate were of this type. The round or oval nucleus was relatively small and distinct in a cytoplasm that tended to be clear. In fresh films there was very little neutral red or none whatever in these cells which were apt to contain a lymphocyte or carbon particles, but after about thirty minutes irregular red granules sometimes appeared and in occasional cells red staining became quite distinct. Often the neutral red was so irregular and granular that it suggested the staining of ingested granular débris.

About one-half of these "hyaline" cells contained carbon but usually only one or a few granules. To test in vitro their phagocytic property there is placed from a syringe at the center of a cover-glass within a 5 mm. wax-pencil circle a minute drop of 1 per cent India ink in saline and to this at once is added an equal amount of exudate from a second syringe. The cover-glass is rimmed with vaseline and at once inverted over a deep well on a concave slide. After thirty minutes at 37° C. the cover-glass is lifted by its corners from the concave slide and placed in the usual way on the dye film. Abundant fibrin threads form but these do not interfere with the phagocytosis or the subsequent examination of the preparation. The picture is remarkable. Both neutral red types take up the carbon but it is the "hyaline" ones that become enormously loaded with the ink particles. These cells are extremely ameboid and tend to agglutinate so that they are found more abundantly entangled in the fibrin toward

the center of the preparation. On this date 10 c.c. of whole blood was injected intraperitoneally and the phagocytic experiment repeated on November 5 with the same results. This exaggerated property of carbon phagocytosis has been demonstrated in several dozen exudates obtained at different times and from different animals. On November 10, 15 c.c. of whole blood was injected intraperitoneally. On November 12, there were about 3 "rosettes" to 1 "non-rosette" to 1 "hyaline." Although the cells were very numerous, only 4 "rosettes" and 5 "non-rosettes" containing carbon were found during one hour. The percentage of the "hyaline" type with carbon has diminished since the last intravenous carbon injection but carbon-containing cells of this type are much more numerous than of either of the other varieties.

Origin of the Phagocytes in Exudates Produced by Injections of Trypan Blue, India Ink and Whole Rabbit's Blood

The origin of the three types of peritoneal exudate cells has been investigated by an examination of tissues and organs from normal, trypan blue, and India ink rabbits, first, in fresh preparations supravitally stained, and second, in paraffin sections of supravitally stained tissues.

Examination in Fresh Preparations of Tissues Supravitally Stained.

Peritoneal Serosa. Peritoneal scrapings made with a knife are placed on a cover-glass ringed with vaseline which is at once inverted on the dye film. Seventy-two hours after the injection of 5 c.c. of India ink there are in the scrapings abundant placques which consist of many or a few cells and in these are seen all cell shapes varying from the flattened non-irritated cell to cells that are almost perfectly spherical, but still attached to other cells. The cytoplasm is finely granular with indistinct cell outline while the nuclear membrane of the nucleus which tends to be relatively large is very distinct. By far the most conspicuous feature is the large and uniform content of very minute carbon particles. In some cells the carbon dust is massed somewhat at one side of the nucleus where the cytoplasm is denser, while in others it is scattered diffusely throughout the cytoplasm. The serosal cells in fresh preparations contain no neutral red but after a half-hour the denser portions of the cytoplasm may become slightly tinged.

The peritoneal surfaces are scraped after second and third injections of 20 c.c. of 2 per cent trypan blue. The serosal cells contain only small amounts of the dye or none at all.

On November 18, 1924, the splenectomized animal described above was chloroformed and peritoneal scrapings were examined in supravital films both before and after incubation with carbon. The phagocytic experiment with carbon was made in the same way as in the case of the exudate. The serosal cells contained no carbon and did not phagocytize carbon in vitro. The other characters of these cells have been described by Cunningham.[7, 8]

Lymph Nodes. Bits of organs and whole lymph nodes are kept in Locke's solution until minute snippings can be teased to dust-like particles in a solution made by adding 1 to 4 drops of 0.5 per cent neutral red to 10 c.c. of saline. A cover-glass is applied to the preparation in the neutral red solution. The second method of examination consists of expressing the "juice" from the lymph node on a cover-glass and at once inverting it on a dye film. The expressed "juice" of glands from normal animals shows such enormous numbers of the "rosette" type that entire fields consist almost entirely of this type. In the teased preparations the "rosette" cells in places are attached and have an average size larger than the lymphocytes which do not react to the dye. The neutral red spherule is in almost all of the cells smaller than in the peritoneal exudate "rosettes." In some oblong cells the neutral red focus may be seen to lie at the side of the nucleus, midway in the cell. Phagocytic ones are very rarely seen. Unlike the lymphocytes the nuclei of these cells present an irregularity of outline. Besides the lymphocytes and "rosette" cells there are occasional larger ones with abundant heavy neutral red granules. In the lymph nodes of ink-injected rabbits there are only occasional carbon-containing phagocytes. Most of these are found toward the capsule of the gland, are large and may or may not contain irregular neutral red particles. In rabbits stained with trypan blue the lymph node preparations are the same as the normal except that there are occasional large phagocytes filled with coarse dye granules which tend to take up the neutral red to assume a purplish color. The carbon-marked and trypan-blue containing cells appear to correspond to the "hyaline" type of phagocyte seen in the peritoneal exudates. The "non-rosette" type is present in scant numbers.

Spleen. In normal uninjected rabbits the "rosette" cells have never been observed in large numbers in material obtained by splenic puncture under ether, by puncture after removal of the spleen from the body, or by teasing snippings of pulp or of the splenic lymph nodules. The "non-rosettte" cells are fairly abundant in normal rabbits. In rabbits being injected intraperitoneally with whole rabbit's blood the "rosette" cells are much more numerous. In the India ink rabbits the numerous carbon-marked phagocytes may show irregular neutral red staining or none at all. In the trypan blue rabbits there are numerous large phagocytes containing irregular granules purple in color from imbibition of the red dye by the trypan blue granules.

Liver. Teased preparations of the ink-injected rabbits in the neutral red-saline solution show heavy masses in the sinusoids consisting of carbon-laden cells. These cells take up little of the dye but at the periphery of these masses mononuclear cells with scattered red granules are seen in small numbers. The sinusoidal endothelial cells show neutral red granules beyond the ends of the nuclei. In rabbits stained with trypan blue there is a much more distinct granulation in endothelial cells which takes on a purple color in the dye films. In normal rabbits irregular neutral red particles may be demonstrated in an occasional endothelial cell.

Bone-Marrow. The less mature myelocytes may have the neutral red granules grouped in a way that suggests the "rosette" cells, but these masses are usually not spherical. In the normal rabbit positive identification of "rosette" cells has not been possible. In the India-ink rabbits the bone-marrow becomes quite dark and large ink-containing phagocytes as well as polynuclear leucocytes with carbon are present. The former show a few coarse particles of neutral red.

Examination in Paraffin Sections of Tissue Supravitally Stained.

It soon became apparent in examining teased bits of tissue that, in order to obtain contact with the dye, it was necessary to destroy to a very large extent cell relations. In fact observation of the fresh tissue proved entirely inadequate for an exact determination of the origin of the cells reacting to neutral red. The method for imbedding tissue supravitally stained is one previously developed by the author[14] for the preservation in paraffin sections of the cytoplasmic granules giving the peroxydase reaction to benzidin. The tissue

stained supravitally in toto is fixed for 12 hours in a Zenker-formol solution consisting of 15 c.c. of 40 per cent formaldehyde and 85 c.c. of Zenker's fluid without acetic acid. It is then cut into pieces not to exceed 3 mm. in thickness and transferred to Zenker's fluid without acetic acid for 12 to 24 hours. Bits of the tissue are then placed in pure absolute acetone for 1 hour (2 changes), in benzol for 20 minutes and in 52°C. paraffin for 20 minutes. Sections of the desired thickness are cut and attached to the albumin-coated slides by allowing them to dry overnight at room temperature. To stain, the paraffin is removed with xylol (10 seconds) and pure acetone (10 seconds). After immersion in water (5 seconds) the slide is stained very lightly with hematoxylin (Harris without acetic acid) for about 5 seconds and immersed in tap water (5 seconds). The section is then dehydrated with pure absolute acetone for 10 seconds, at once immersed in xylol for 10 seconds and mounted in balsam. To limit the action of the acetone and xylol to these times the slides are stained singly and the solutions run over them from a dropping bottle. A 0.05 per cent aqueous solution of methylene blue may be substituted for the hematoxylin.

To stain the tissue supravitally in toto the animal is bled completely from the carotids under light ether anesthesia and saline solution saturated with neutral red (Gruebler) is at once injected directly into the tissues. About 0.5 c.c. is injected into the liver or spleen by inserting a fine needle 2 cm. from the edge of the organ and slowly forcing out the dye after carrying the needle to within a few millimeters of the margin of the organ. The injection is repeated in 15 minutes. The inguinal, iliac, substernal and axillary lymph glands are injected by inserting the needle into the substance of the gland and forcing out the dye until the gland is red and distended. After about 30 minutes the injected tissues are removed, covered with cotton moistened with saline and after another 30 minutes at room temperature placed in the Zenker-formol. It is always advisable to imbed all the available tissue, for the contact with the dye is irregular. Similar results have been obtained in rabbits and guinea pigs. A larger number of guinea pigs have been examined and for this reason some of the best reactions have been obtained in them. The nuclei of the cells that are killed by the injection stain red, and no observations are made within the areas in which the cells are injured.

Lymph Node. In the normal lymph gland both "rosette" and "non-rosette" cells are abundant and both are found especially in the sinus ramifications toward the hilum. Few neutral red cells occur within the larger masses of lymphoid tissue such as the secondary nodules although they are present at the periphery of these structures. That the dye has penetrated this dense lymphoid tissue is shown by the presence there of occasional neutrophiles or blood vessel endothelium with dye granules. The anastomosing lymph sinuses when collapsed consist of connecting cells which as usual are not to be differentiated from reticular cells. They are larger than lymphocytes and tend to be elongated or oval. The nuclei are not spherical and frequently an actual hof is present. These are the cells that react to the dye. In areas in which the dye penetration is good with several dozen "rosette" cells present in one microscopic field the idea of the very great number of these cells is obtained. The flat cells with scant cytoplasm that line the peripheral and larger sinuses are usually devoid of dye but the reaction may be identified in definite endothelium lining medullary sinuses. These channels are apt to be broadened by the injection and may be followed for considerable distance in longitudinal section. Where they are surrounded by more compact masses of lymphoid tissue no lumen can be seen. With this arrangement rows of reticular cells are seen, each with its spherule of neutral red. Here and there the larger of the reticular cells present a perfect rosette appearance. In these larger cells one may occasionally observe two or more foci of fine granules but it is usually within the lumina of the larger sinuses that cells with several foci of minute granules occur where there are also cells with the fine granules distributed throughout the cytoplasm. All transitions are present from the reticular cells with a single small collection of fine granules to those with rosettes and with numerous scattered red granules. There is, however, unquestionably a tendency for the neutral red granules to remain in foci. In rabbits given intravenous injections of ink these cells are devoid of carbon but large carbon-containing phagocytes are present in small numbers in the sinuses and especially in the fibrous capsule of the gland. These carbon-phagocytes show neutral red granules irregularly distributed when they are brought into contact with the dye. In rabbits injected with carbon suspension subcutaneously the reticulo-endothelium of the regional lymph nodes contains carbon particles but

these cells do not usually become engorged to form great ink masses like those seen in the liver or spleen of rabbits intravenously injected. The small foci of fine neutral red granules are present in young reticular carbon-containing cells of the subcutaneously injected rabbits. The ink granules are not grouped in rosette forms by these cells. It is advisable to stain six or more lymph nodes for both neutral red and carbon reactions vary. The lymph nodes draining such ink foci offer perfect evidence that the reticular cells are reached by the lymph although the channel that they line cannot be seen in the usual section.

The view that any distinction between the lymphocytic endothelium and the reticular cells is arbitrary was first advanced by Aschoff and Kiyono.[15] They hold that the two are indistinguishable and function in the same way. They make no distinction as regards function or morphological features between the reticulo-endothelial apparatus of the spleen and that of the lymph nodes although one carries blood and the other lymph. Their conclusions are based largely on results obtained with vital dyes. Maximow,[1] on the other hand, considers the reticular cells to be a type entirely distinct from the vascular endothelium.

Spleen. An intravenous injection of 1 c.c. of India ink several days before the test is helpful in defining the structures of the spleen where even under the best conditions of fixation and staining, or even in specimens with injection mass in the blood vessels, the vasuclar network in its fine ramifications in the pulp and lymphoid tissue is not easy to distinguish. In places the endothelium of the vessels contains fine red granules, but mononuculear cells which lie within and between the well-defined sinuses and which have a conspicuous fine red granulation scattered diffusely in the cytoplasm are especially abundant. These numerous phagocytes with a very fine diffuse granulation appear to be free or semi-detached. Some of them contain carbon, red corpuscles or leucocytes. In the guinea pig Kurloff bodies in the lymphocytes are likely to stain supravitally and to be conspicuous. In these preparations the neutral red granulation does not aid greatly in distinguishing these cells from myelocytes, but in control oxydase stains myelocytes are not abundant. The granulation of these mononuclear phagocytes appears to be brought out only by a relatively high concentration of dye, but as in the case of other tissues cell injury is shown by nuclear reaction to

the neutral red, and the cytoplasm of such injured cells does not show the granulation. These numerous free cells have much the same appearance as the larger attached endothelial cells, but it is highly important to recognize the ease with which mononuclear cells circulating in the blood are arrested in the spleen. The "rosette" cells are relatively infrequent and the young forms of "rosette" cells with single foci of neutral red granules have not been seen in the pulp or at the periphery of lymphoid nodules where dozens are present in single oil-immersion fields in the lymph nodes.

Liver. It may be necessary to examine several blocks from different parts of the injected bit of tissue in order to find an area that has reacted well. The Kupffer cells and other endothelial cells lining the sinusoids which have a notable amount of cytoplasm may contain neutral red granules irregular in shape and size, but in well-stained foci others of these cells contain no dye. Some of the granules have the irregular appearance of ingested material that has taken up the stain but others are fine and regular. The mild irritation of a preliminary intravenous injection of 1 c.c. of India ink causes some increase in the size of the endothelium without obscuring it by overloading with carbon. In the elongated cells the dye granules may be present only at one end of the nucleus but more often are situated at both ends. In the larger endothelial cells and the Kupffer cells the dye granules are almost always scattered about in the cytoplasm much like the ink particles. Free within the sinusoids large phagocytes with carbon and dye in abundance are found.

Discussion

The polymorphonuclear leucocytes, lymphocytes, and the desquamated serosal cells which appear in peritoneal exudates are quite readily identified in fresh preparations supravitally stained with neutral red and Janus green. Following injections of whole rabbit's blood into the peritoneum there appear in the peritoneal cavity three types of mononuclear phagocytes which have for convenience of description been designated the "hyaline," the "rosette," and the "non-rosette" types according to the amount and arrangement of neutral red in them.

The "hyaline" cells vary in size from about 20 microns to very large cells. The nuclei tend in the larger ones to be relatively small

and to lie excentrically in the glassy cytoplasm. These cells are actively ameboid and frequently contain ingested lymphocytes and especially amorphous material that takes up more or less of the neutral red. Some cells have irregular dye granules while others have none. They become more abundant after many injections of whole blood. They are clearly defined by incubation with carbon suspension since they adhere to the fibrin threads where they form agglutinated masses of phagocytes containing enormous quantities of ingested ink particles. In rabbits that have the vasuclar endothelium marked by the intravenous injection of India ink more than one-half of this type of cell appearing in the peritoneal cavity after the introduction of whole rabbit's blood may contain one or two carbon particles, but cells with great masses of carbon such as those present in the capillaries of the spleen and liver rarely appear in the exudate. When whole rabbit's blood is injected into rabbits stained vitally, a considerable number of the cells of this type contain the coarse trypan blue granules. The reactions of this type of peritoneal phagocyte to carbon and to some extent to neutral red are essentially the same as those of the blood vascular endothelium seen in fresh preparations and in paraffin sections of tissue supravitally stained. The presence in these cells of carbon and trypan blue granules is evidence favoring their vascular origin.

The "rosette" and the "non-rosette" types were originally described by Sabin and co-workers.[4, 9] By them the "rosette" cell was considered to be the monocyte or transitional cell of the peripheral blood and to be derived from a mesenchymal cell in the splenic pulp while the "non-rosette" cell was regarded as the "macrophage" of reticulo-endothelial origin. The observations of the author in fresh tissue and in paraffin sections of supravitally stained tissue of normal and splenectomized rabbits indicate the lymph node origin of the "rosette" cell. In paraffin sections of supravitally stained lymph nodes the reticular lymph vascular endothelial cells lining the medullary lymph sinuses and forming the complex anastomosing channels about the hilum of the gland and in the periphery of the follicles of lymphoid tissue contain cytoplasmic foci of dye granules or dye granules in actual wreath form. In some of the larger cells there is more than one focus of dye granules. Within the larger lymph sinuses there are free cells with dye granules in the form of rosettes and with dye granules scattered about in the cytoplasm. There is,

however, a tendency for the granules to remain more or less circumscribed in certain portions of the cytoplasm. That is, in the attached reticular cells the dye-reacting cytoplasm may progress to the "rosette" form but the rosette formation and any further dissemination of the supravitally staining granules usually takes place after the cells separate or at least after they assume a semi-detached position in the sinuses. It appears therefore that phagocytes originating in the lymph nodes as well as those arising from the blood vascular endothelium may present neutral red granules scattered diffusely in the cytoplasm, but that the granules are always scattered in the blood vascular ones while they tend to remain in foci in those of lymph node origin. There is therefore satisfactory evidence that the neutral red granules may become dispersed in the cytoplasm with a change from rosette to non-rosette form.

In rabbits injected simultaneously with ink into the ear vein and with whole rabbit's blood into the peritoneal cavity the relatively large percentage (10 to 20 per cent) of "rosette" and "non-rosette" cells with phagocytized carbon is regarded as evidence that these cells are transported in part at least through the blood stream. The percentage of carbon-containing polymorphonuclear leucocytes is about the same. At the end of 4 days less than 1 in 100 of the "rosette" cells contains carbon, although ink granules may be present in one-half of the "hyaline" cells at this time. Since the blood vascular endothelium is carbon marked, the tendency for carbon-containing "hyaline" cells to persist and be present at a time when few granular leucocytes and few mononuclear neutral-red types contain carbon is significant. In rabbits moderately stained with trypan blue a small number of cells with coarse dye granules appear in the exudates produced with whole blood and when supravitally stained such cells take up neutral red so that the dye granules assume a purple color. In the lymph nodes of the animals vitally stained to a moderate degree with two or three injections of trypan blue there are a few large phagocytes in the capsule and in the peripheral sinus of the gland which contain coarse dye granules but the abundant "rosette" cells of the lymph node at this time contain none. In the adult rabbits no evidence was obtained indicative of a form of the "rosette" cell younger than the reticular endothelium which is present in the lymph node and which freely undergoes mitotic division to form its own kind.

After completion of this article but before its submission for publication, Sabin and co-workers [16, 17] have given a much more detailed presentation of their work. The illustrations are especially helpful. In Figs. 5, 6, and 13 of Plate I, portraying clasmatocytes, of the article by Sabin, Doan, and Cunningham [16] there appear to be none or very few neutral-red granules in the cell cytoplasm. In their earlier communication the clasmatocytes or macrophages were described as follows: "They were usually large cells, 15 to 30 micra in diameter. These cells always reacted intensely to neutral red; the aggregations of the stain varied greatly in size and color (slight red to deep maroon) and were scattered irregularly, without any pattern, throughout the cell." From an analysis of the text and illustrations the inference is made that their clasmatocytes correspond to the author's "hyaline" type. They apply the term "wandering endothelial phagocyte" to the clasmatocyte and regard it as the derivative of the vascular endothelium. It is therefore chiefly in regard to the monocyte or "rosette" cell that their view differs from that of the author, since they state (p. 158): "Thus we consider that the weight of evidence points to two separate strains of phagocytic cells of connective tissue: clasmatocytes, which are of endothelial origin and come into the blood stream only occasionally and abnormally, and monocytes, which are a constant type of blood cell, arising, largely in the spleen, but also a specific cell of the diffuse connective tissues, where they both arise and function." The examination of lymph glands made by Cunningham, Sabin, and Doan [17] gives results emphatically different from those of the author. Examination of the fluid obtained by puncture of mesenteric lymph glands gave them results as follows (p. 259): "Over 90 percent of the cells obtained from such a puncture, especially if the pipette does not rupture any blood vessels, are unquestionably members of the lymphocytic series. The other 10 percent are made up of a few clasmatocytes, a few polymorphonuclear leukocytes, and an occasional myelocyte. . . . In no experiment where blood vessels were not ruptured by the puncture did we find any typical young monocytes." Lymph nodes have not been punctured with the fine capillary pipettes used by these observers but it is scarcely conceivable that cells free from the "rosette" type could be obtained unless a very fine capillary entered a lymph follicle. In juice expressed from inguinal, iliac, and axillary lymph nodes, cells with single focal areas

of neutral red granules including rosettes at times number one-third of all cells in the preparation. In paraffin sections, as described, these cells are very numerous and correspond to the reticular endothelium.

Cunningham, Sabin, and Doan reach the final conclusion: "that there are two fixed-tissue stem-cells from which all the blood cells arise. From the endothelium the red blood-cells and the clasmatocytes are formed, and from the reticular cell there is formed a primitive stem-cell which in turn gives rise to the polymorphonuclear leucocytes, the monocytes, and the lymphocytes." The impression has been gained by the author from observations on adult tissues that the phagocyte of the "hyaline" type which appears usually to have been designated as macrophage, histiocyte or clasmatocyte, arises from blood vascular endothelium, while the phagocyte presenting the fine nuetral red granules, often in the form of a rosette, takes its origin from the reticular lymph vessel endothelium which is in the adult tissues a cell distinct from the lymphocytes and younger lymphoblastic cells at the germinal centers. The evidence supporting the lymph-node origin of the "rosette" type is considered to be conclusive. In the preparations from which these conclusions are drawn the lymphocytes show not the slightest trace of neutral red. With a dissemination of the granules and loss of the characteristic wreath appearance the older cells of this type are not so readily identified in supravital stains, and they assume the form designated by the author in this paper as the "non-rosette" form and by Sabin and co-authors as the "macrophage" cell. That all of the cells of the "non-rosette" group of the author are of this type however seems doubtful since the "hyaline" form of phagocyte may show scattered neutral red granules.

Observations in animals with their vascular endothelium carbon-marked and in animals vitally stained to a moderate degree with trypan blue indicate that the "hyaline" form of the author arises from the blood vascular endothelium, but the proof is less direct than that by which the "rosette" cells are traced to the lymph nodes. In the spleen the predominant phagocytic cell is one with scattered fine neutral-red granules, and, since an investigation of the cells of this organ is essentially a study of the mononuclear phagocytes of the blood, a detailed discussion of it here will not be undertaken. It may be stated merely that it corresponds to the "non-rosette" type of the author which has a double origin. So far as

can be determined removal of the organ does not influence the character of the phagocytes appearing in peritoneal exudates.

Conclusions

1. By the method of peroxydase staining with benzidin, reacting mononuclear phagocytes are not demonstrable in the peritoneal exudates of rabbits.

2. By the method of supravital staining the mononuclear phagocytes of peritoneal exudates may for the purpose of description be divided into three groups: "hyaline" cells with a variable affinity for neutral red; "rosette" cells with neutral red in wreath form; and "non-rosette" cells with dye granules scattered diffusely in the cytoplasm.

3. By in-vitro incubation with carbon, by the intravenous injection of carbon suspension and by vital staining with trypan blue, the "hyaline" type may be differentiated from the other cells of the exudate. In paraffin sections of supravitally stained tissue in normal and carbon-injected animals, this type of cell reacts much like the reticular blood-vessel endothelium. This evidence suggests the vascular origin of the "hyaline" type.

4. By the examination of fresh tissue and paraffin sections of fixed tissue supravitally stained, satisfactory evidence of the origin of the "rosette" type from the reticular lymph vessel endothelium is obtained.

5. The "non-rosette" type with its diffuse neutral-red granulation is a mixed group of double origin. By the methods employed all mononuclear phagocytes of this type in the exudates appear to be either "hyaline" cells of probable blood-vascular origin, which may have diffuse neutral red granules, or "rosette" cells in which the granules have become disseminated.

6. The lesser phagocytic activity for coarse carbon particles of the lymph-node phagocytes and the tendency for neutral-red granules to remain localized in foci in them offer a basis for the differentiation of a lymphatic and of a blood-vascular or hemal type of mononuclear phagocyte. Although the free phagocytes may undergo mitosis, the fixed-tissue source is the blood- and lymph-vessel endothelium. The endothelial origin of the peritoneal phagocytes

agrees with the conclusion previously arrived at by the author [5] in regard to the mononuclear phagocytes of the peripheral blood.

7. As a means for the identification of cells, further evidence of the value of their vital reaction to chemical or physical agents is seen in the application of neutral red solution to the living mononuclear phagocytes of peritoneal exudates. To obtain exact data in regard to the origin of the cells it is essential to fix the neutral red in the tissues for examination in paraffin sections.

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STUDIES ON FILTERABLE VIRUSES *

I. CULTIVATION OF VACCINE VIRUS

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One of us [1] in a preliminary note has reported the successful cultivation of vaccine virus in vitro. The medium employed was a tissue culture of rabbit testis. The virus was successfully carried through a maximum of nine transfers, representing a total time of fifty-four days in the incubator. The virus showed a definite increase on comparing the first and third generations of two of the cultures. We wish to report below further work along the same general lines.

Growth of Virus in Normal Tissue

In the work referred to above each culture was started with pieces of fresh, vaccine-infected testis. In view of Wolbach's [2] experience with the cultivation of Rocky Mountain spotted-fever virus, in which he gained the impression that only cells originally infected in the animal, or descendants of these cells, contained the virus in cultures, we thought it of interest to attempt to discover if the same held true of vaccine virus.

For this purpose pieces of normal testis were soaked for five minutes in a suspension of glycerinated virus-infected testis, that had been kept in the ice box for seventy-one days, after which time of course no cells could be expected to survive. These pieces of testis were then covered with drops of normal rabbit plasma, diluted with two parts of Ringer's solution, incubated for five days, transferred, reincubated, etc., as described in the preliminary note. The fourth generation of this culture was tested on a rabbit's cornea and found markedly positive macro- and microscopically. This virus, VC 6 A, was carried through 36 transfers (Table I). The experiment would appear to show that the growth of this virus, unlike that of spotted fever, does not take place only in the originally infected cells, but

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occurs either in or near normal tissue cells. Other experiments carried out in a somewhat different way also confirm this conclusion. In these experiments pieces of normal testis were covered with drops of normal plasma to which had been added small amounts of a heavy undiluted suspension of glycerinated, vaccine-infected testis. Here again, after several transfers, virus could be demonstrated and shown to be growing in all of the cultures (VC 10 — Table II — represents one of these cultures).

Numerous investigators in studying vaccinia in animals and man have attempted to demonstrate the virus in the blood of the infected animal, but always without success until quite recently. Ohtawara,[3] by using intratesticular inoculations with comparatively large amounts of blood, has shown conclusively that the virus does occur in the blood stream and remains there several days following inoculation on the skin with vaccine virus. This was true not only of rabbits, but in one instance of a human. This being true, the virus in the blood must occur in very small numbers, for ordinary methods, such as corneal and skin inoculations with blood, are always negative. In view of these facts we felt that it would be of interest to use as an inoculating material the plasma of an infected rabbit, for should our cultures prove positive on corneal or skin inoculation with this plasma as the sole source of the virus, it would show that definite growth of the virus had taken place presumably in the absence of primarily infected cells. Therefore, cultures were set up using the plasma of a rabbit inoculated (cornea, skin and testis) with vaccine virus five days previously, and pieces of normal rabbit testis. The cultures were incubated and transferred in the usual way. The third generation, inoculated on the cornea of a rabbit, was strongly positive macroscopically and showed many typical Guarnieri bodies microscopically.

Duration of Virus Growth in Artificial Medium

In the former piece of work, vaccine virus was successfully cultivated for a total period of fifty-four days, at which time the work was discontinued. We decided to attempt to discover how long we could cultivate the virus. A culture, VC 6 A, was started June 25, 1924, using normal plasma and pieces of normal rabbit testis that had been soaked for five minutes in a heavy emulsion in Ringer's solution of a seventy-one-day-old, glycerinated, virus-infected rabbit

testis. This culture was incubated, transferred, and tested in the usual way (Table I).

TABLE I

Growth Record of Vaccine Virus Culture, VC 6 A

Date		Generation	Result of testing
6/24/24	Started	I	
7/1	Transfer	II	
7/7	"	III	
7/11	"	IV	Cornea +
7/15	"	V	
7/21	"	VI	
7/25	"	VII	
7/30	"	VIII	
8/5	"	IX	Cornea +; Skin +
8/11	"	X	
8/15	"	XI	
8/20	"	XII	
8/27	"	XIII	
9/3	"	XIV	
9/10	"	XV	
9/15	"	XVI	
9/19	"	XVII	
9/24	"	XVIII	Cornea +; Skin +
9/29	"	XIX	
10/3	"	XX	
10/9	"	XXI	
10/15	"	XXII	
10/22	"	XXIII	
10/28	"	XXIV	Cornea o; Skin o; Testis + Emulsion of this rabbit's testis inoculated on another rabbit's cornea, skin and testis; all were +.
11/3	"	XXV	
11/7	"	XXVI	
11/13	"	XXVII	
11/19	"	XXVIII	
11/25	"	XIX	
12/1	"	XXX	
12/5	"	XXXI	Cornea o; Skin o
12/11	"	XXXII	Cornea o; Skin o; Testis o Emulsion of this rabbit's testis inoculated on another rabbit's cornea, skin and testis; all were o.
12/16	"	XXXIII	
12/22	"	XXXIV	
12/27	"	XXXV	
1/2/25	"	XXXVI	Cornea o; Skin o; Testis o Tested 3/6/25 for immunity as described below.

As will be seen from the above table, the fourth generation, inoculated on a rabbit's cornea, was positive macro- and microscopically; the ninth generation tested on the cornea was positive and a dilution equivalent to $\frac{1}{4600}$ of the culture injected intradermally was positive. The twenty-fourth generation was negative on the cornea and skin, but positive macroscopically in the testis. This testis was removed, an emulsion made and inoculated on another rabbit's cornea, skin and testis. All of the latter were positive macroscopically and the cornea and skin were positive microscopically. This result with the twenty-fourth generation may have been due to decreasing virulence of the virus, or to a diminished number of organisms, for the amount inoculated into the testis was several times that inoculated on the cornea and skin; however, the possibility of the development of a tissue affinity on the part of the virus due to continued cultivation in testicular tissue should be taken into consideration. The thirty-first generation, inoculated on the cornea and skin, was negative macroscopically. The thirty-second generation, inoculated on the cornea, skin and testis, was negative macroscopically in all; however, the testis was emulsified and inoculated on another rabbit's cornea, skin and testis. All these were negative macro- and microscopically. The thirty-sixth generation was negative on the cornea, skin and testis; this rabbit was tested for immunity on March 6, fifty-seven days after inoculation. This was done by inoculating on both corneas, skin and testis with an emulsion of glycerinated, vaccine-infected testis, and also on the skin with commercial vaccine virus. Both corneas and both testes were positive macro- and microscopically; the skin both with the commercial virus and the testis emulsion showed what was similar to an accelerated take in the human; that is, a marked take at the end of twenty-four hours, which was fading at seventy-two hours. Another rabbit, previously inoculated with positive results, and a normal rabbit were inoculated at the same time with both viruses; the revaccinated animal also showed an accelerated take while the reaction in the normal did not reach its height until the fourth day. Therefore it would appear that the rabbit inoculated with the thirty-sixth generation showed evidence of immunity as far as the skin was concerned. We carried out this experiment in this way as we felt that there was a possibility either that the virus might be present in too small amounts to give a positive take but in sufficient amounts to confer immunity, or that it

had lost its infecting power but had preserved its immunizing power. To sum up this cultivation experiment, the virus was demonstrated to be present in the fourth, ninth, eighteenth and twenty-fourth generations, covering a period of one hundred and thirty-two days of incubation; it did not give a positive take with the thirty-first, thirty-second and thirty-sixth generations; but the last apparently brought about some skin immunity on inoculation into a rabbit. The thirty-sixth generation represents a period of one hundred and ninety-eight days' incubation.

One experiment was undertaken to determine the amount of virus present in the ninth generation. The tissue of one of the cultures was weighed (5.9 mg.), ground with sand and 1.0 c.c. of Ringer's solution and centrifuged. Various dilutions of the supernatant fluid were made. One-tenth c.c. of each of these dilutions were injected intradermally into a rabbit. A dilution representing $\frac{1}{4600}$ of the culture (0.0013 mg. of culture) gave a positive skin test. Unfortunately this represented the maximum dilution and so the exact limit of positivity was undetermined.

Proof of Multiplication of Virus

We have felt all along that, while experiments, such as the one just mentioned and the one where we started with infected plasma, proved that the virus actually multiplied in our cultures, we should endeavor to devise some method whereby the amount of virus present in any particular culture could be determined. Obviously corneal and skin inoculations with scarification are unsatisfactory, as the amount of inoculated material that actually comes in contact or remains in contact with the tissues long enough to infect, must vary tremendously; furthermore the depth and number of scarifications are other variable factors. Intradermal injection seemed to offer a method wherein the amount injected and site of injection could be most accurately controlled, and this method has proved most satisfactory.

The technique of titration is as follows: the whole culture (tissue and plasma) is ground up with 1.0 c.c. Ringer's solution in a sterile test-tube using a sterile glass rod and sterile sand; it is then centrifuged at moderate speed for five minutes, the clear or slightly opalescent supernatant fluid pipetted off and the desired dilutions of the latter made; these dilutions are injected intradermally in 0.1 c.c.

(rarely 0.2 c.c.) amounts using a tuberculin syringe and a fine (No. 26 gauge) needle.

This method of titration was carried out with culture VC 10 and the results are given in Table II. The original culture was started with pieces of normal rabbit testis and normal plasma to which had

TABLE II

Growth and Titration of Vaccine Virus Culture, VC 10

Generation	Date	Split	Titration			Taking Skin Doses Contained per Culture	Virus Content Relative to Original Inoculum
			Date	Amt. Used	Result		
I	11-25-24		11-25-24	1 culture*	0.2 c.c undil. + 0.1 c.c. undil. −	$2\frac{1}{2}$*	1
II	12- 1-24		12- 5-24	1 culture*	0.2 c.c. undil. −	<$2\frac{1}{2}$*	<1
III	12- 5-24						
IV	12-11-24		12-16-24	2 cultures	0.1 c.c. 1-50 + 0.1 c.c. 1-100 not done	250	10
V	12-16-24	Yes					
VI	12-22-24		12-27-24	2 cultures	0.1 c.c. 1-200 + 0.1 c.c. 1-400 −	1,000	800
VII	12-27-24						
VIII	1- 2-25	Yes	1- 8-25	4 × $\frac{1}{2}$ culture	0.1 c.c. 1-400 + 0.1 c.c. 1-800 −	2,000	1,600
IX	1- 8-25						
X	1-14-25	Yes					
XI	1-20-25		1-26-25	2 cultures	0.1 c.c. 1-3200 + 0.1 c.c. 1-6400 not done	16,000	51,000

* These two cultures contained originally 2 drops of virus plasma mixture instead of 1 drop. In each instance the "taking skin dose" is calculated on the assumption that only 1 drop was used.

been added such an amount of glycerinated vaccine-infected testis that the plasma virus mixture represented a dilution of about 1:10 of the undiluted testicular suspension. Each piece of testis was covered with one drop of the plasma virus mixture, except for two pieces which received two drops apiece. One of the latter cultures, as soon as clotting had taken place, was removed, ground in 1.0 c.c. of Ringer's solution and titrated on a rabbit; 0.2 c.c. of the undiluted suspension gave rise to one vesicle. The other inoculations were all negative. Hence, this control culture contained a sufficient amount of virus to cause five positive intradermal reactions and all of the

other cultures, with the exception of the second one, which received 2 drops of plasma virus mixture, contained theoretically on the basis of this titration sufficient virus to cause two and a half positive intradermal reactions.

The cultures were then incubated and transferred as shown in Table II. When transferring, the tissue was freed as much as possible from its surrounding plasma. Obviously this method resulted in the loss at each transfer of a certain amount of virus; this loss has not been taken into account when figuring the relative strength of the original culture and the subsequent generations. If the whole culture could have been transferred each time, instead of only the tissue, the virus content of each generation would have been greater than that found. By the term "split" is meant that, as the amount of tissue in a culture became too large for satisfactory growth, this tissue was divided in half as evenly as possible and each half carried on as an individual culture, with the result that each culture of the subsequent generation contained only one half as much virus as before the splitting occurred.

Inspection of the column in Table II labeled "Taking Skin Doses per Culture" reveals the fact that a continuous increase in the virus content per culture took place from the third generation on. The reduction in virus content in the second generation was probably due to the fact that a considerable part of the virus had died, for it is known that incubation of the virus in plasma diminishes its strength rather rapidly. Between the second and fourth generations the virus evidently began growing well, for the fourth generation contained ten times as much virus as the original. From this generation onward multiplication occurred until in the eleventh generation the virus content per culture was 51,000 times that of the original. This figure was calculated as follows:

$$\frac{16{,}000\left\{\begin{array}{l}\text{number of skin taking}\\ \text{doses in 11th generation}\end{array}\right\}}{2.5\left\{\begin{array}{l}\text{number of skin taking}\\ \text{doses in first culture}\end{array}\right\}}\times 8\left\{\begin{array}{l}\tfrac{1}{8}=\text{portion of first}\\ \text{culture in 11th gen-}\\ \text{eration due to}\\ \text{splitting}\end{array}\right\}=51{,}000$$

The average weight of our cultures was 10 mgm. Therefore, in the eleventh generation, $\frac{1}{1600}$ mgm. of the culture contained one taking skin dose, or one gram of this culture would yield 1,600,000 taking skin doses, whereas one gram of the original contained only 250 such doses.

This culture after the eleventh generation was unfortunately contaminated with moulds and had to be discarded.

Location of Virus Growth

There has been throughout the work a question in our minds as to whether the virus grows within the cells, near the cells or at a distance from the cells in the plasma clot with which the tissue is covered. In an experiment reported in the preliminary note, it was shown that a normal piece of testis was infected by being placed in the same clot as an infected piece of testis although a space of 2 or 3 mm. of plasma intervened. In this experiment infection of the piece of normal testis might have taken place through (1) growth of the virus through the plasma, (2) migration of the virus through the plasma, (3) migration of infected cells through the plasma, or (4) transfer of the virus in the serum squeezed out of the clot. In order to throw light on the region of growth, a piece of virus tissue culture was placed in a plate, and covered with plasma, a projection of plasma about 1.0 cm. long being made at one side. After seven days' incubation the tissue and the plasma prolongation were removed separately and each was weighed, emulsified in Ringer's solution and similar dilutions made with regard to the weight of each piece. These various dilutions were then tested on a rabbit. The testis emulsion was positive with a dilution representing $\frac{1}{4000}$ of the whole, whereas the plasma emulsion was negative throughout. This experiment suggests that the growth of the virus takes place in intimate connection with the tissue. Histological investigations of our cultures have failed to throw any light on location of the virus or its morphology, so at present we cannot say whether growth is intra- or extracellular.

Unsuccessful Attempts to Cultivate Virus

It seems worth while to record some of our unsuccessful attempts to cultivate vaccine virus in media other than the one used in the above experiments.

1. Pieces of culture testis from VC 6 A, sixth generation, were cultivated in serum agar. These were transferred every sixth day to fresh medium of the same composition. The sixth transfer was tested on a rabbit's cornea and proved negative macro- and microscopically.

2. A piece of culture testis from VC 6 A, sixth generation, was placed on the side of a 100 × 31 mm. test tube, about 0.5 cm. from the bottom. To this was added normal diluted rabbit plasma, the tube was slanted and the plasma allowed to clot. The tube was then placed upright and 2.0 c.c. of Ringer's solution added. After six days' incubation two drops of the Ringer's solution were transferred to another tube of the same composition, except that normal rabbit testis was used instead of culture testis. Two drops were also added as controls to a tube of Ringer's solution and plasma, and to another of Ringer's solution and normal testis. After five days' incubation the Ringer's solution in the original tubes and the Ringer's solution in the various tubes of the last transfer were tested on rabbits' corneas and all found negative macro- and microscopically.

This experiment was repeated incubating the tubes anaerobically, as well as aerobically, using as virus VC 6 A, ninth generation. After three transfers the fluids in both the anaerobic and aerobic cultures were tested intradermally in a rabbit and were negative.

3. As Carrel and other investigators have shown that chick embryonic tissue can be cultivated in artificial media for long periods of time, we thought this tissue might prove useful for our purposes. In a Carrel[4] "D" plate were placed several pieces of a fourteen day old chick embryo; these were covered with a mixture composed of 1.0 c.c. normal rabbit plasma, 0.15 c.c. chick embryonic juice, 0.15 c.c. emulsion of glycerinated vaccine-infected testis and 0.5 c.c. Ringer's solution. After clotting, 1.0 c.c of a Ringer's solution dilution (1:15) of chick embryonic juice was added. A second plate similar to the first except that no virus was added was set up as a control on tissue growth. The supernatant fluid was removed every three days, the cultures washed with Ringer's solution as recommended by Carrel and fresh chick embryonic juice diluted with Ringer's solution added. Tissue growth in both plates was good. At the end of 19 days' incubation (6 washings) the supernatant fluid, plasma and tissue of the virus culture were each tested separately by intradermal injections on a rabbit and all were found negative.

4. As embryonic juice is known to be a growth stimulant, it seemed advisable to try its effect on the virus using normal rabbit tissue rather than embryonic chick tissue. The cultures were set up in 100 × 13 mm. test-tubes, a piece of normal rabbit testis being

placed in the bottom. To each tube was added a mixture containing 0.25 c.c. 1:5 Ringer's solution dilution of virus culture VC 10, tenth generation, ground up in 1.0 c.c. Ringer's solution, 0.25 c.c. chick embryonic juice and 2.5 c.c. Ringer's solution. One tube was incubated aerobically, the other anaerobically. At the end of six days 0.25 c.c. of the fluid from the bottom of each tube was transferred to a tube containing similar amounts of normal testis, chick embryonic juice and Ringer's solution, and the tubes were incubated aerobically and anaerobically as before. At the same time some of the fluid from each of the original tubes was tested intradermally on a rabbit and found to be negative. The cultures were transferred after three days and again at the end of six days; at this time the fluid in each tube was again tested intradermally on a rabbit with negative results. After two more days' incubation, the piece of testis in each tube was emulsified in the fluid part of the culture and these emulsions were injected intradermally into a rabbit, and found to be negative.

Summary

1. Vaccine virus can be cultivated in tissue cultures composed of normal tissue; the presence of previously infected, living cells is not necessary.

2. Vaccine virus was successfully cultivated in an artificial medium for one hundred thirty-two days. After one hundred ninety-eight days, the virus was not demonstrable by the usual methods, but apparently gave rise to a certain degree of skin immunity as tested by revaccination.

3. A method has been devised by means of which the virus content of any culture can be determined with a fair degree of accuracy. Using this method, the eleventh generation of one culture was shown to contain 51,000 times as much virus as the material from which it was started.

4. Our results suggest that the virus grows only in close proximity to the cells. Whether this growth takes place intra- or extracellularly cannot be stated at this time.

5. Various other methods for the cultivation of vaccine virus were tried without success.

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STUDIES ON FILTERABLE VIRUSES *

II. CULTIVATION OF HERPES VIRUS

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The results obtained in our attempts to cultivate vaccine virus, as reported in the preceding paper, seemed to justify the expectation that this method might be applied with hopes of success to the cultivation of others of the so-called filterable viruses. For this purpose we have used herpes virus and a note on the cultivation of this virus is given below.

The strain, "M," used was very kindly furnished us by Dr. E. W. Goodpasture. The material for the first culture, H 18, was obtained from a rabbit inoculated intracerebrally with glycerinated herpes-infected rabbit brain. The animal was killed in the convulsive stage and the brain removed aseptically. To each small piece of brain tissue was added a piece of normal rabbit testis, for we felt that the growth of the brain tissue might be insufficient to encourage growth of the virus. The plasma was obtained from the herpes-infected rabbit. At each transfer fresh normal rabbit testis and plasma were added, as with our vaccine virus cultures. The cultures were "split" whenever too much tissue for good tissue growth accumulated.

As a source of virus for our second and third cultures, H 19 and HC 3, we used rabbits' testes that had been inoculated forty-eight hours previously with an emulsion of the brain of a herpes-infected rabbit. In the fourth culture, HC 4, we used the testis of a rabbit that had been injected intratesticularly forty-eight hours previously with an emulsion of HC 3, tenth generation.

The complete records of these cultures with the results of testing are given in Table I. In each instance the macroscopic results were controlled microscopically. A positive diagnosis was not made unless we found typical, intranuclear inclusions in epithelial cells of the cornea or in the interstitial cells of Leydig of the testis, a location for the latter first described by Goodpasture and Teague.[1]

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As a control, to rule out the question of survival, one of the original plates was kept in the incubator without transferring. After fourteen days rabbit inoculation showed that the virus was dead, or at least present in insufficient amounts to give positive tests. This particular experiment was repeated with the same result. Therefore, we can safely say that incubation of the virus at 37.5° C. in plasma for two weeks either kills the virus or diminishes it to such an extent that positive takes cannot be obtained. From Table I it will be seen that in all the experiments the minimal incubation period at the time of first testing the cultures equaled or exceeded fourteen days.

We were able to cultivate one culture, HC 3, for a minimal total period of fifty-four days. No tests were made between the tenth and fifteenth transfers so that we do not know at what point the virus died. It seems possible that the virus may have survived for a longer period than that demonstrated.

TABLE I

Culture and Inoculation Records of Herpes Virus Cultures

Culture	Date	Generation		Result of Testing
H 18	7/5/24	Started	I	
	7/11	Transfer	II	
	7/15	"	III	
	7/21	"	IV	
	7/25	"	V	7/30 Cornea +
	7/30	"	VI	
	8/5	"	VII	
	8/11	"	VIII	
	8/15	"	IX	
	8/20	"	X	
	8/27	"	XI	9/3 Brain 0; Cornea 0
	9/3	"	XII	
	9/10	"	XIII	
	9/15	"	XIV	
H 19	7/10/24	Started	I	
	7/15	Transfer	II	
	7/21	"	III	
	7/25	"	IV	7/30 Cornea +
	7/30	"	V	
	8/4	"	VI	
	8/9	"	VII	
	8/11	"	VIII	
	8/15	"	IX	8/20 Cornea 0
	8/20	"	X	

TABLE I (*continued*)

Culture	Date	Generation		Result of Testing
HC 3	9/10/24	Started	I	
	9/15	Transfer	II	
	9/19	"	III	9/24 Cornea +; Testis +
	9/24	"	IV	
	9/29	"	V	
	10/3	"	VI	10/9 Cornea +
	10/9	"	VII	
	10/15	"	VIII	
	10/22	"	IX	
	10/28	"	X	11/3 Cornea +; Testis +
	11/3	"	XI	
	11/7	"	XII	
	11/13	"	XIII	
	11/19	"	XIV	
	11/25	"	XV	12/1 Cornea 0; Testis 0
	12/1	"	XVI	
HC 4	11/5	Started	I	
	11/7	Transfer	II	
	11/13	"	III	11/19 Cornea 0; Testis 0
	11/19	"	IV	
	11/25	"	V	12/1 Cornea +
	12/1	"	VI	
	12/5	"	VII	
	12/11	"	VIII	
	12/16	"	IX	Cornea 0

As yet no definite proof of multiplication can be presented, for no method of titration has been evolved that does not necessitate the use of an excessive number of animals.

In one culture, HC 4, while the third generation was negative, the fifth generation was positive, which suggests that multiplication must have taken place.

The reason for the death or loss of virulence of the virus is not yet clear. One fact has been noted which may have some bearing on this question: namely, after several transfers the fibroblasts in the fresh normal testis added each time grow very poorly or not at all. It may be that the virus in growing kills or injures the fibroblasts, and the poor growth of these in turn results in the eventual death of the virus. The fact that herpetic intranuclear inclusions occur in fibroblasts, as well as in epithelial cells, suggests that the virus has some affinity for connective tissue cells. With HC 3 one culture showed no fibroblastic growth, whereas another of the same generation showed very good growth. These two cultures preserved

these same characteristics for six weeks during which time they were transferred seven times. In this period each culture was "split" twice and the daughter cultures of each preserved the same characteristics. No virus could be demonstrated in either by the usual methods. It seems probable that either we had an attenuated form of the virus to account for this inhibition or the tissues themselves had developed some substance inhibitory to the growth of fibroblasts, possibly akin to the bacteriophage.

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STUDIES ON ENDOTHELIAL REACTIONS

IX. THE FORMATION OF RETICULUM IN THE LESIONS OF EXPERIMENTAL TUBERCULOSIS IN RABBITS *

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Introduction. Investigations on the nature of the reticulum in the lesions of tuberculosis have recently been given impetus by the studies of Miller (1923) and Long and Miller (1924). In the past, Mall's (1891, 1896) work on the normal reticulum was not followed up with any marked diligence until Maresch modified the Bielschowsky technic of silver impregnation to apply to it, whereupon his much less complicated method stimulated a series of investigations by Rössle and his pupils, Kon (1908), Russakoff (1909), and Yoshida (1909) — all of whom studied the reticulum of various organs under normal and pathological conditions. Their conclusions were that this form of fibrous tissue is a direct product of cellular activity, that it may present true reticular structure, or may be a reticular thickening in sheet-like membranes (particularly in epithelial organs), and that it probably represents a precollagenous form of fibrous connective tissue. Neuber (1912) subsequently made a study of the cardiac reticulum under normal and pathological conditions. All these investigators agree that this material is entirely independent of the elastic fibrous tissue, and present convincing proof of this assertion. Russakoff made a painstaking investigation of the reticulum in pulmonary tubercles, and Miller has supplemented his work with an equally meticulous study of the reticulum in the tubercles in a child's lung. He agrees that elastic tissue and

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reticulum are unrelated, and affirms his belief that the latter presents an early stage in the formation of collagenous connective tissue, under certain circumstances.

The histogenesis of reticulin fibrils is still very poorly understood; the earlier writers believe it to be formed within endothelial cell processes, and Kon, studying its production in human embryos, states that it is laid down in the fourth month of development as granular linear structures in these processes, the granules becoming fused to form threads in the fifth or sixth month. Miller takes exception to this theory, pointing out that the fibers form so complicated and abundant a network that their formation could scarcely be explained in this manner. That there is a definite relationship between cellular activity and fibril production seems to be agreed; Corner (1920) has stressed the almost universal association of endothelial cells and reticulin fibrils and concludes that the latter are usually produced by the capillary endothelium; the association of the reticuloendothelial cells with reticulum formation has been known since Mall's first paper; and it seems that the fibroblast may also be closely associated with reticulum, particularly in the medullary rays of the kidney. Whether the reticulin forming these fibrils differs from that laid down near endothelial cells remains to be seen. No reticulum is seen in the glomerular tuft under ordinary circumstances, although I have occasionally seen it in rabbit kidneys.

White and Smith (1924) have recently suggested that there is a relationship between fibrin formation and the production of reticulin, that the former might be converted into the latter by the agency of the endothelium. They point out that recent investigation has proved that reticulin fibrils are formed outside of cells, appearing in the embryonal body fluid before cells of any sort are demonstrable in their proximity. They note that both Hertzler and Baitsell (1915, 1916) have shown that fibrin may be converted into connective tissue fibrils "which cannot be distinguished, either in their structure or tinctorial reactions, from the fibrils of reticulum and white fibrous tissue." They apply these theories to the formation of tubercle reticulum, believing that this may be produced by the fibrin at the center of the tubercle. They state that reticulin is probably a gelatinous material until fixed by the various coagulant reagents used for this purpose, when it takes on the familiar thread-like form, an hypothesis also advanced by Rössle and Yoshida.

The foregoing review naturally leads to the question: What is reticulin? Is it purely an artefact, or does it exist as a gelatinoid network before fixation? The term "reticulin" is applied to the substance forming the fibrillar network which is called "reticulum." Is every fibrillar substance that takes the Bielschowsky silver impregnation (exclusive of nervous tissue) composed of reticulin? That it is not an artefact is indicated by the experiment of Rössle and Yoshida, who bruised and pinched freshly removed lymphnodes before fixation and later found the reticulum correspondingly distorted and compacted into a felt-like material in the sections cut after fixation. Whether or not the silver impregnation is exactly specific for reticulin we cannot say, but it would appear to be so.

Thus we are faced by several questions: Does the tubercle reticulum represent a direct transformation of fibrin into reticulum? Is the agency of cells necessary for its production? Is it laid down within those cells as Russakoff claimed, or outside of them as Miller, and White and Smith suggest? Does reticulin become further elaborated into collagen? To attempt an answer to these questions, tubercles were produced in rabbits, representing lesions in various stages of the progressive development of the process, and sections from these were stained specifically for fibrin, reticulum, and collagen.

Experiments. Seven rabbits were inoculated intravenously with 0.2 mg. per kgm. body weight of a bovine strain used in the experiments described in the preceding paper of this series. (Foot, 1923.) One rabbit was killed after one week, one died of intercurrent infection at eleven days, and tuberculosis killed one at fourteen, two at sixteen, and one at eighteen days, thus affording a graded scale of lesions. Two rabbits from another experiment were added to this series. They had both received the same dose of the same strain intravenously eighteen days before death.

A second series, composed of older lesions, was selected from material collected by Dr. Austin, head of this department, who kindly placed it at the writer's disposal. In this set, stages were obtained representing five weeks (1), six weeks (2), eight, nine, fifteen, seventeen, and twenty-three weeks (1 each). These were all produced by the subcutaneous inoculation of 5 mg. per kgm. of the same bovine strain.

Technic: The tissue was fixed in Zenker's fluid, imbedded in paraffin and cut to five microns; in each case, sections were made from the lung, liver, spleen, and kidney. For the study of fibrin, Mallory's phosphotungstic acid hematoxylin and the Gram-Weigert stain were used; for that of collagen, Weigert's modification of the Van Gieson stain and the phosphotungstic acid hematoxylin technic; and for that of the reticulin, the writer's modification of the Bielschowsky-Maresch silver impregnation. (Foot, 1924.) Harris' hematoxylin was substi-

tuted for Weigert's iron-hematoxylin in the latter and the results were very much improved, owing to the greater range and variety of color.

By means of this combination of methods the production of fibrin could be detected and studied; collagen fibers could be distinguished as soon as they appeared; and reticulin and collagen fibrils could be demonstrated and distinguished one from the other in the silver impregnations. Sets of routine hematoxylin-eosin sections were prepared in each case, as a check on the general topography of the lesions and tissues; in all instances the sections were practically serial.

Fibrin and Reticulin: That there is no immediate or direct transformation of fibrin into reticulin is readily ascertained in this experiment.

No fibrin is found in the seven-day tubercles, although it is occasionally present in vascular clots. The eleven-day rabbit died of fibrinous pneumonia, pleuritis, and pericarditis; the fibrin stains well in the exudate, and some is found in the pulmonary and hepatic tubercles that have developed as a result of the specific inoculation, but it is centrally located and quite unlike reticulin in its appearance and tinctorial reaction. In the twelve-, fourteen-, and one of the sixteen-day rabbits, fibrin is found in small quantities in the liver tubercles, usually centrally; it is very variable in the pulmonary tubercles, appearing in small quantities in the lumina of capillaries at the periphery of the lesions in one case only — the two-week rabbit. In the more advanced lesions of the second series of subcutaneously inoculated rabbits it is occasionally present, but inconstantly and in no relation to the production of reticulin or collagen.

The fibrin network has not the faintest resemblance to reticulum; it is coarser, more translucent, its threads run a far straighter course and have nodal thickenings at the intersections, and it is usually found at the center, rather than at the periphery of the tubercles. Comparing serial sections, one stained for fibrin, the other for reticulin, one finds that there is no similarity in appearance, or in distribution, between the fibrinous network and the reticulum. The one is coarse, central, and straight; the other fine, peripheral, and curly or waving. The fibrin might stain faintly with acid fuchsin in the Van Gieson stain, but it is never impregnated by the silver. For these reasons it would seem almost certain that there is no direct relationship between the two and no immediate transformation from one to the other. Furthermore, there is a well-developed reticulum in the pulmonary tubercle at one week, although no fibrin is demonstrable. Later lesions show the same thing, the production of fibrin and of reticulin appear to be independent processes.

Reticulin and Collagen (*First Series of Rabbits*). In the first series of lesions it is readily ascertained that the reticulum is best studied in the liver tubercles, where it does not appear in quantity until about the fourteenth day. In pulmonary tubercles there is already a delicate reticulum at the end of a week. In the spleen the investiga-

tion is very difficult and unsatisfactory, on account of postmortem autolysis, the presence of splenic reticulum, and peculiar precipitates in the pulp. The kidney is already too rich in reticulum and the lesions too scanty to render its study very profitable.

Liver. The liver tubercles first show a very delicate reticulum after eleven days have elapsed. This seems to be continuous with the sinusoidal reticulum, which is very scanty in rabbits as compared with that of human livers. The fibrils are often beaded and they are branching and short. They are most definite at the periphery of the tubercle and more plentiful in lesions occurring near Glisson's capsule than in those at some distance therefrom. It is impossible to say that these fibrils are produced within cells; they lie at the periphery of endothelial phagocytes and are usually continuous with neighboring sinusoidal fibrils. At first they are more delicate in the tubercles than in the sinusoids, but after two weeks they become so coarse and prominent that one may readily locate tubercles by looking for dark spots in the field. The sinusoidal reticulum becomes strikingly thicker and more prominent in the neighborhood of the tubercles. It has just been stated that reticulin fibrils do not run within the cytoplasm of endothelial cells, but there is one exception: they can be demonstrated penetrating that of the giant-cells and running through them between the nuclei. This could be explained by the assumption that these syncytia are formed by the fusion of endothelial phagocytes which had formed fibrils at their periphery prior to fusing, which would include them in the common cytoplasm.

The gradual transformation of reticulin into collagen is strongly indicated in all the tubercles of this first series; the older the tubercle, the pinker become the coarser fibrils. As the silver impregnation colors collagen old-rose, and the superimposed Van Gieson stain renders them scarlet, this change is easily followed.

Summarizing this first series of lesions, we find that reticulum is formed before either fibrin or collagen; that there is no evident relationship between fibrin and reticulin, whereas there is a striking parallelism between the formation of reticulin and collagen; and that the process is best studied in its inception in the hepatic tubercles produced by intravenous injection of tubercle bacilli. We also see that a definite causal relationship between cells and reticulum cannot be established by this method of procedure, although the universal presence of endothelium in the neighborhood of the reticulum strongly indicates that the former plays some part in the production of the latter.

Second Series of Rabbits. The series of older lesions, produced by subcutaneous inoculation and ranging from five to twenty-two weeks between inoculation and death, present several features differing from those of the first series. The exact age of the systemic

tubercles is necessarily indefinite, as they result from infection from the primary subcutaneous tubercles; the subcutaneous tubercles may bring about allergic phenomena which could influence reticulum formation (see Long and Miller, 1924); and the development of the systemic tubercles is more difficult to follow stage by stage. The following facts are noted: the liver ceases to be the best organ in which to study reticulum formation, as this is not only very variable, but many of the hepatic tubercles show no reticulum whatsoever. The spleen continues to be poor terrain for observation, and the kidney varies according to whether it be infected or not — if tubercles are produced, there is a great deal of dense reticulum formed, more than in the other organs. The most instructive organ in this series is, therefore, the lung; in considering it, it will be best to review the results of observation on both series of rabbits, commencing at one week and continuing stage by stage through the twenty-two week rabbit.

Lung. There are, as we know, two types of pulmonary lesion in this disease: one starting within the alveolar wall, and one in which there is intraalveolar exudate. The progress of the two lesions may be traced along one or the other of these two lines, or they may be found to be combined, which is usual. In the first case one notes a local multiplication of endothelial cells near the alveolar capillary; there is a nodular thickening composed of ovoid epithelioid cells. A delicate reticulum forms immediately and its fibrils are very intimately connected with the endothelial cells, but they are directly continuous with the preëxisting pericapillary reticulum and appear to be prolongations of its fibrils rather than independently formed products of cellular activity, or cytoplasmic processes (Fig. 1). This growth continues and the tubercle bulges into the neighboring alveolus, or alveoli (Fig. 2). At the same time, an exudate of endothelial phagocytes appears in the air-space of the alveolus and if the epithelial lining remain in tact no reticulum is seen among the cells of this exudate; if the epithelium become necrotic, however, the tubercles break into the air-spaces and grow there, carrying their reticulum with them and sending branches thereof among the cells of the exudate (Figs. 3 & 5). There is no indication that these cells produce reticulum that is independent of, and unconnected with, that of the alveolar wall. The latter becomes destroyed, its capillaries disappear, and the alveolar boundaries are obliterated. If caseation supervene, the reticulum is largely destroyed (after a short period of brisk proliferation) and remains as short, thick, rod-like masses in the caseous material. The reticulum of the alveolar walls bounding such caseous areas becomes greatly increased and thickened in situ (Fig. 4). There is a variant of this process: near veins it is noted that there is little thickening of the alveolar wall, but an exudate of discrete endothelial phagocytes fills each alveolar sac. Reticulum appears to run directly from the wall of the vein into the exudate and to ramify among its cells, without awaiting the destruction of the alveolar wall. The latter process follows eventually, and the vessel becomes surrounded by a sheath-like mass of

epithelioid tissue, which is liberally supplied with reticulum. If the vein be the site of a tuberculous thrombus (as often happens in this experiment) the reticulum runs into this thrombus, as well as out into the perivascular tissue (Figs. 6 & 7). Either of these processes may result in the complete wiping-out of the pulmonary topography over considerable areas and the laying down of extensive fields of reticulum (Fig. 8).

The invasion of the intraalveolar exudate may become very extensive (Fig. 5) so that the alveolar boundaries are scarcely recognizable, having lost their capillaries and epithelium and being interspersed by fields of reticulated exudate. After eighteen days of development, one notices that the coarser reticulum begins to take on a reddish color and this increases steadily in its intensity until many of the fibers are quite red, indicating that they have been converted into collagen. In the older lesions, at about fifteen weeks, one finds that the formless fields of reticulum are becoming converted into networks of rather dense, chiefly collagenous fibers, and that the interstices are filled with lymphocytes, plasma cells, and endothelial phagocytes, all of which are separate from one another and from the fibrils of the matrix. In other words, a typical scar has been produced, although this interpretation of its production differs from the usual conception of fibroblastic proliferation, which is notably absent. This idea implies destruction, collapse and shrinkage, and proliferation of the preëxisting reticulum with conversion of the latter into collagen, rather than invasion by fibroblasts and formation of fibrous tissue through their agency.

The first complete scars noted in this series are seen in the lung of the seventeen-week rabbit (Fig. 9). They are composed of loose complexes of thick, collagenous fibers, loosely scattered cells of various types, and vessels of moderate caliber and thin walls, some of them more or less completely thrombosed by tuberculous tissue which is well preserved, provided with a reticulum, and not necrotic. The coarse collagen fibers are continuous with finer reticulin fibers, and these with a delicate reticulum of interlacing fibrils. (Cf. Miller, 1924.) Transitions of reticulin to collagen are observed all through these fields; a given fiber may be red at one end and black at the other, or blackish nodes of reticulin may be found imbedded in red collagen fibers. Fig. 9 represents this condition, the use of red light for the photography renders the collagen gray, while the reticulum stands out as black lines, dots, or dashes on this gray background. This may give one a clue as to the steps in the process of transformation from reticulin to collagen. Although there is a good deal of collagen present after eighteen days, it is not conspicuous in the silver sections until a month or more has elapsed, and then the transformation is incomplete, much reticulum remaining unconverted. Only the silver impregnation can demonstrate this satisfactorily (Fig. 10).

A study of the reticulum of tubercles in organs other than the lung, in the second series, adds little or nothing to the impressions already gained from the first.

Discussion. The results of this experiment throw considerable light upon the formation of reticulum in tubercles, but they have not cleared up the mystery of its histogenesis to the extent one might have anticipated. It will be necessary to experiment with

the formation of reticulin in tissue cultures, in connection with the mononuclears of the blood, along the lines taken by Margaret Lewis in her recent brilliant experiments (Lewis, 1925), before we can say just what rôle the endothelium plays in the formation of this substance. Such an experiment will form the basis for a subsequent paper. It can be said, however, that one gains the distinct impression, while examining the slides of this series, that the new reticulum is merely a prolongation of the old; that it represents a growth by accretion, as it were, rather than a laying down of reticulin in cell processes. It may be, and probably is the result of secretions of fibroblasts or of cellular derivatives of the vascular or reticulo-endothelium: a process analogous to the precipitation of a fibrin network through the activity of fibrinogen, which is now known to be contained in largest quantities in those organs that are richest in endothelium. That there is no direct transformation of fibrin into reticulin is sufficiently indicated in the preceding text. The assertions of earlier writers as to the conversion of reticulin into collagen is amply borne out by these experiments; it would seem that there can be little doubt as to the truth of their beliefs.

Conclusions

1. The histogenesis of the reticulum is not satisfactorily solved by this method of study; cellular derivatives of the endothelium seem to play an important part in forming reticulum, for they are always present in its vicinity — but they do not appear to produce it by intracellular activity.

2. It is indicated that newly formed reticulum is a product of pre-existing reticulum, as it is always continuous with it.

3. Fibrin plays no direct part in the production of reticulin.

4. Reticulin is apparently converted directly into collagen, but the manner in which this transformation is effected has yet to be explained.

5. Caseation and reticulin formation are incompatible; after a preliminary proliferation in areas of early caseation, the reticulum is destroyed.

6. The presence of tubercles in an organ appears to stimulate the growth and to cause a coarsening of the normal, preëxisting reticulum in their immediate vicinity. This might be interpreted as a forerunner of the production of newly-formed reticulin fibrils.

7. More reticulum is produced in those tubercles occurring in or near portions of an organ already rich in that tissue. Conversely, it is scanty in those occurring at some distance from the stroma of the organ.

8. The importance of collapse in the process of scar formation in chronic tuberculosis of the lung is emphasized in sections impregnated with silver.

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DESCRIPTION OF PLATES LVI-LVII

Plate LVI

Fig. 1. A tubercle at one week, bulging from either side of an interalveolar septum into the neighboring air-sacs. Thickening of adjacent alveolar wall. The reticulin fibrils are already abundant and there is thickening of the original reticulum. The newer fibers are continuous with the older.

Fig. 2. Larger tubercle at eleven days. Alveolar boundaries are beginning to be lost, there is great thickening of the preëxisting reticulum and a tendency for new fibrils to invade the intraalveolar exudate.

Fig. 3. An area which, in a hematoxylin-eosin section, would pass for a conglomerate tubercle. Note that the alveolar reticulum, although dispersed and indefinite, still maintains enough of its original topography to suggest about nine air-sacs. There is invasion of the exudate by newly-formed reticulum. Eighteen days after inoculation.

Fig. 4. Similar to Fig. 3, but shows the usual marked thickening of the original reticulum about a caseous area, as well as the formation of reticulum in the exudate. Twelve-day lesion.

Fig. 5. The alveolar boundaries are hard to make out, the walls thickened, the capillaries gone. The reticulum is more diffuse and branching and does not confine itself to the pericapillary tissue. Six-week lesion.

Fig. 6. A reticulated intravenous thrombus composed of epithelioid cells; eighteen days after inoculation. The continuity of the intravascular with the perivascular reticulum is afforded by the original reticular ring within the wall of the vein, from which the new reticulum seems to spring. The perivascular alveoli are invaded by a fibrous network of reticulin which is in far closer relationship to the original vascular reticulum than it is to the endothelial phagocytes, which seem rather to have become enmeshed in its fibrils than to have produced them.

Plate LVII

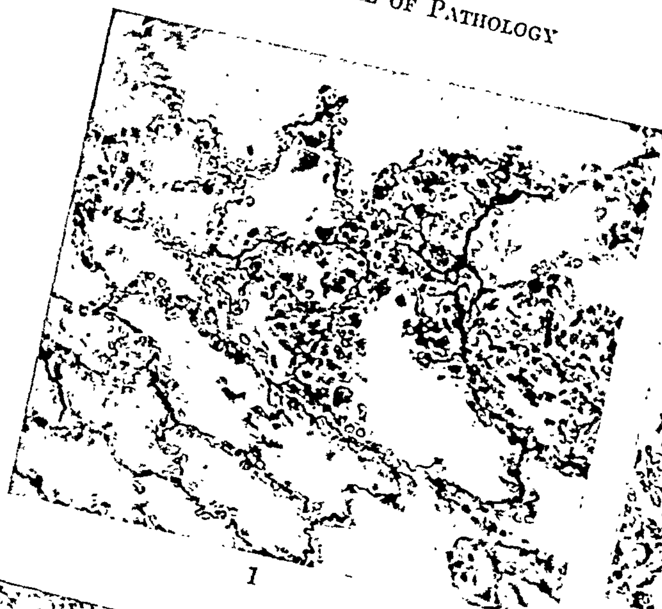
Fig. 7. Completed tuberculous thrombus in a large vein fifteen weeks after subcutaneous inoculation. Both intra- and perivascular reticula are very coarse.

Fig. 8. The earliest type of diffuse scarring by collapse of air-sacs, loss of topography, and diffuse proliferation of the reticulum. This is not an interlobular septum. Fourteen days after inoculation.

Fig. 9. Presumably a similar area, fifteen weeks after subcutaneous inoculation. Alveoli destroyed, vessels increased in size, much of the reticulin converted into collagen, which photographs gray. Note the nodal, or beaded appearance of the surviving reticulin, which is imbedded in collagen. This gives some clue as to the manner in which the latter replaces the former.

Fig. 10. A scar after seventeen weeks. It is mostly collagen, with comparatively little reticulin remaining. The alveoli are destroyed, or collapsed and converted into "acini." There are many lymphocytes and plasma cells lying enmeshed in the fibrous tissue.

Note: All these photomicrographs show a magnification of 350 diameters; they are all taken from sections stained by my modification of the Bielschowsky-Maresch method; the exposure time is the same in every case; therefore they are strictly comparable with one another. They were all taken with red light, Wratten filter "F," in order to subdue the details of the cellular elements and emphasize the reticulin fibers, as well as to tone down the bright-red collagen fibrils so that they would photograph gray, instead of black. This differentiates them from the reticulum which stains deep black in these sections.



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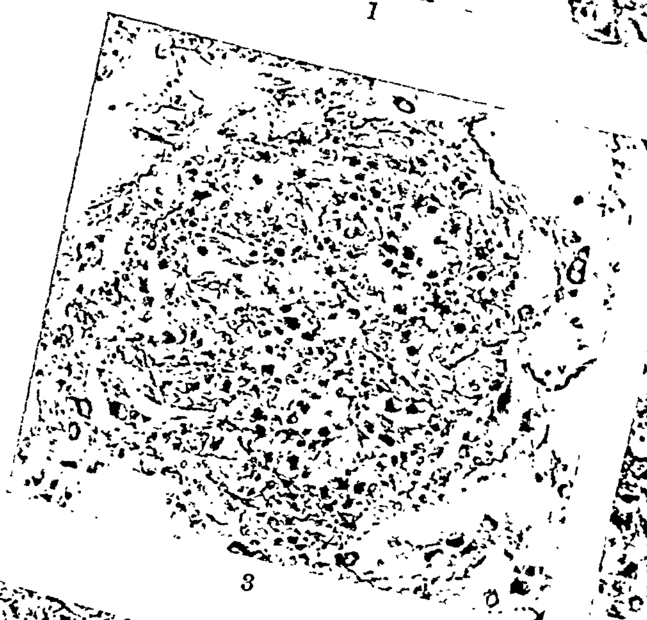

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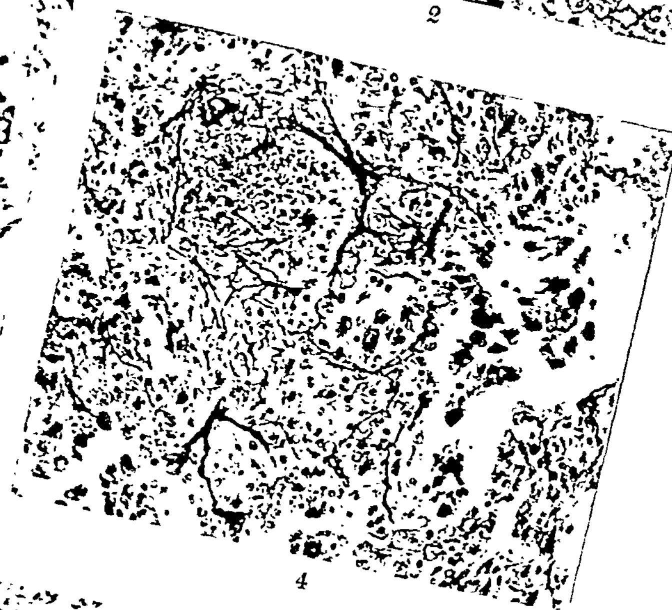

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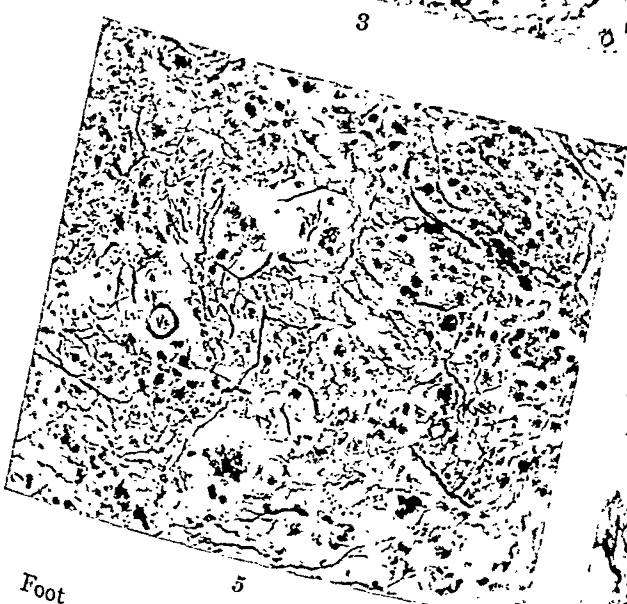

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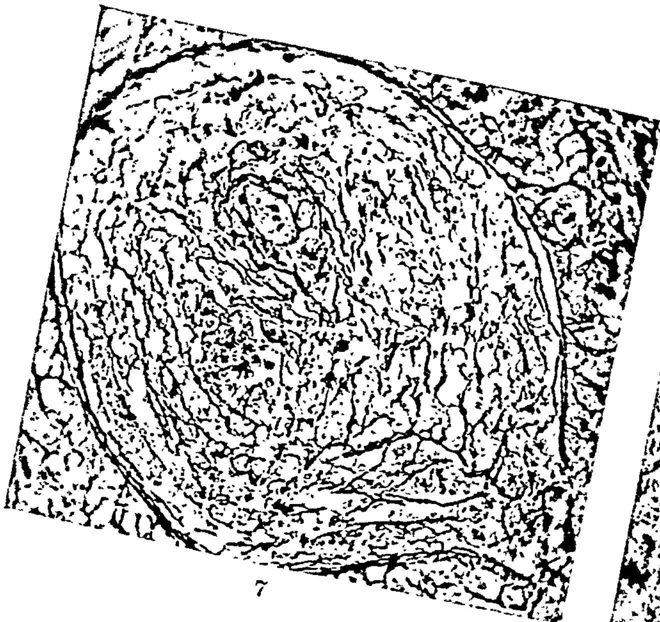
6

The formation of reticulum



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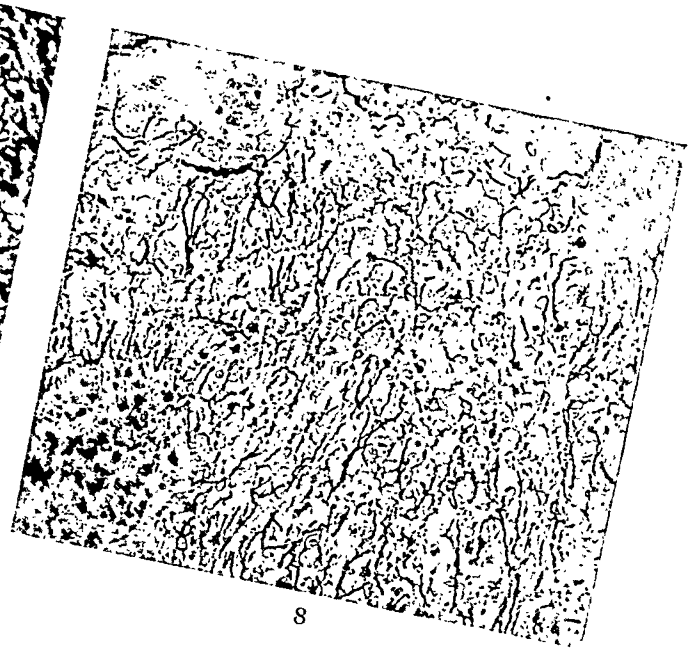

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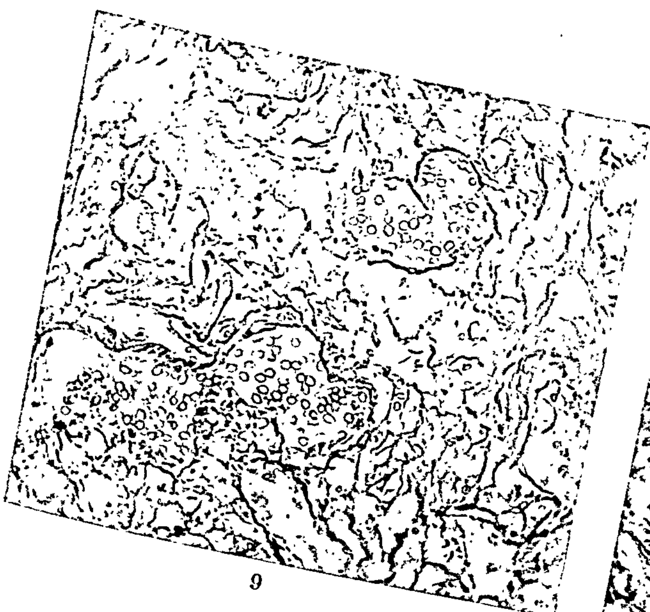

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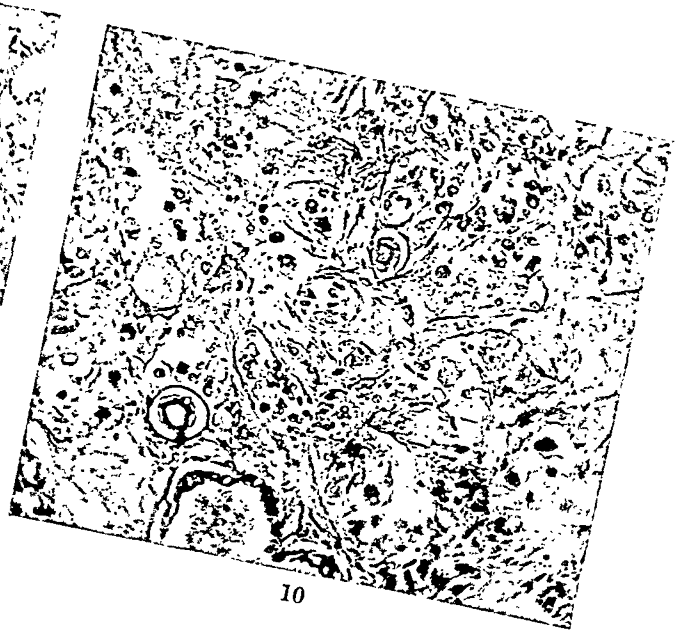

10

Foot

The formation of reticulum

THE STATE OF THE CARDIAC MUSCLE IN HYPERTROPHY AND ATROPHY *

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Introduction

It is generally considered that in hypertrophy of the heart there is increase in the size of some of the muscle fibers and in atrophy there is a decrease in size of many of the fibers, but this view is not sufficiently supported by accurate study. The recent literature shows a disposition to accept the hypothesis that in the human heart, hypertrophy is due almost entirely to an increase in the size of the fibers and that the converse is true in atrophy. The study here presented deals with this question on the basis of measurements of the transverse diameters of large numbers of fibers in the normal, hypertrophic and atrophic human heart. The hearts examined were selected so as to avoid confusion due to coincident disease of the myocardium. The nuclei were counted in many fields, but without regard to the number of fiber units (the part of the fiber between two intercalated discs), since it is admittedly difficult to be sure, in all instances, of the boundaries of these units. The study deals with large numbers of fibers so as to reduce the error of random sampling. The data obtained have been carefully reduced on a mathematical basis.

Literature

Many of the older writers, including Vogl, Köllicker, Lebert, Hyrtl and Rokitansky, were of the opinion that in hypertrophy of the heart the muscle is the seat of a hyperplasia or multiplication of fibers rather than simple hypertrophy. As late as 1911 Wideroe appears to hold this view. Included among those who have held the view that there are both hyperplasia and hypertrophy are Wedl, Foerster, Friedrich, Paget, Rindfleisch, Zielonko, Orth and Adami. Kaufmann and Aschoff both express the view that the process is

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one of pure hypertrophy. These views are also supported by the admirable studies of Tangl and of Goldenberg.

The subject of atrophy in general has been carefully studied by Bradley and his collaborators, who regard the process as autolytic in character. Jamin, in an experimental and morphologic study of skeletal muscle, concludes that simple atrophy such as that due to inactivity is different from a degenerative atrophy in the absence of retrogressive changes other than the atrophy itself. In his concept the term atrophy implies a reduced functional capacity. He finds as the result of inactivity a reduction in size of the fibers. This is in conformity with the work of Durante and of Schmidtman, who, however, also find an occasional large or hypertrophic fiber. All these investigators have found an increase in the number of nuclei, a feature emphasized in most textbooks on pathology. There is still question, however, as to whether the information yielded by the study of skeletal muscle is applicable to cardiac muscle. As to the relations between nuclei and muscle, the work of Collier and of Edens is of especial importance. The former finds that in the hypertrophic heart the ratio between area of nucleus and area of cytoplasm is essentially the same as in the normal heart. Edens determined the ratio of purine and total nitrogen in cardiac muscle with the idea that the method will furnish an estimate of the ratio of nuclear and cytoplasmic substance. In the normal and hypertrophic heart the ratio is about the same but in the atrophic heart the purine nitrogen is significantly increased, thus indicating a relative increase of nuclear substance.

In our study an attempt was made, with the aid of Dr. N. W. Ingalls, to reconstruct the heart muscle fibers by serial section and wax plates. This was found to be impracticable and the method given below was adopted.

Method

Hearts were selected so as to be free from other conditions than those to be studied. Thus, the picture is not confused with coincident inflammatory or destructive changes. Age was regarded as of importance and the hearts were from patients about 30 years of age. The normal heart was from a patient 32 years of age, the hypertrophic heart from one 36 years of age, the atrophic heart from one 28 years of age. All were males and of medium stature. The hyper-

trophic heart was from a patient with chronic glomerulonephritis, the other two from patients with pulmonary tuberculosis. The organs were freed of adventitious tissue, weighed fresh and the volume determined by water displacement in the same manner in all three cases. Blocks were made from corresponding positions near the base of the left ventricle. This situation was selected because it gave the best picture of longitudinal sections of the fibers. The blocks were fixed in 4 per cent formaldehyde, embedded in paraffin, cut in serial sections 5 micra thick including the entire thickness of the heart wall, and stained with hematoxylin and eosin.

A filar micrometer ocular was used in the study. The size of the field selected, on the basis of convenience, was uniformly 0.15 by 0.2 millimeters as determined by the stage micrometer. In each field the number of longitudinally cut fibers was counted. In order to avoid duplication only those fibers parallel to the long axis of the marked field were included. Similarly, the number of nuclei was counted by fields rather than in reference to fiber units because of the difficulty of being certain of the position of the intercalated discs. For this purpose the 16 mm. objective was employed. Knowing the thickness of the section each field represents 0.00015 cubic millimeter. The transverse diameter of longitudinally cut fibers was measured by the graduated drum of the filar micrometer. Here the 4 mm. objective was used. Each 0.1 division of the drum corresponds to 3.5 micra, but in order to save labor the measurements are referred to in multiples of the drum divisions. In each field one large fiber and one small fiber were measured. As will be seen from the tables large numbers of fields were counted and large numbers of fibers measured.

Results

Number of fibers per field

	Maximum	Minimum	Average
Normal heart	19	7	11.5 ± 0.021
Atrophic heart	17	7	10.8 ± 0.023
Hypertrophic heart	11	5	7.4 ± 0.015

A comparison of the averages for the normal heart and hypertrophic heart shows an apparently significant difference, but between the normal and atrophic hearts the difference is not so great. An analysis of the figures is given in Table 1 and shown graphically

in Fig. 1. In the normal heart there is a fairly wide distribution of the various numbers per field with the maximum of fields in those which contain 12 fibers, close to the average of 11.5. In the hypertrophic heart the maximum of fields is in those which contain 8 fibers slightly above the average 7.4. There are no fields containing

TABLE I

Number of fibers per field

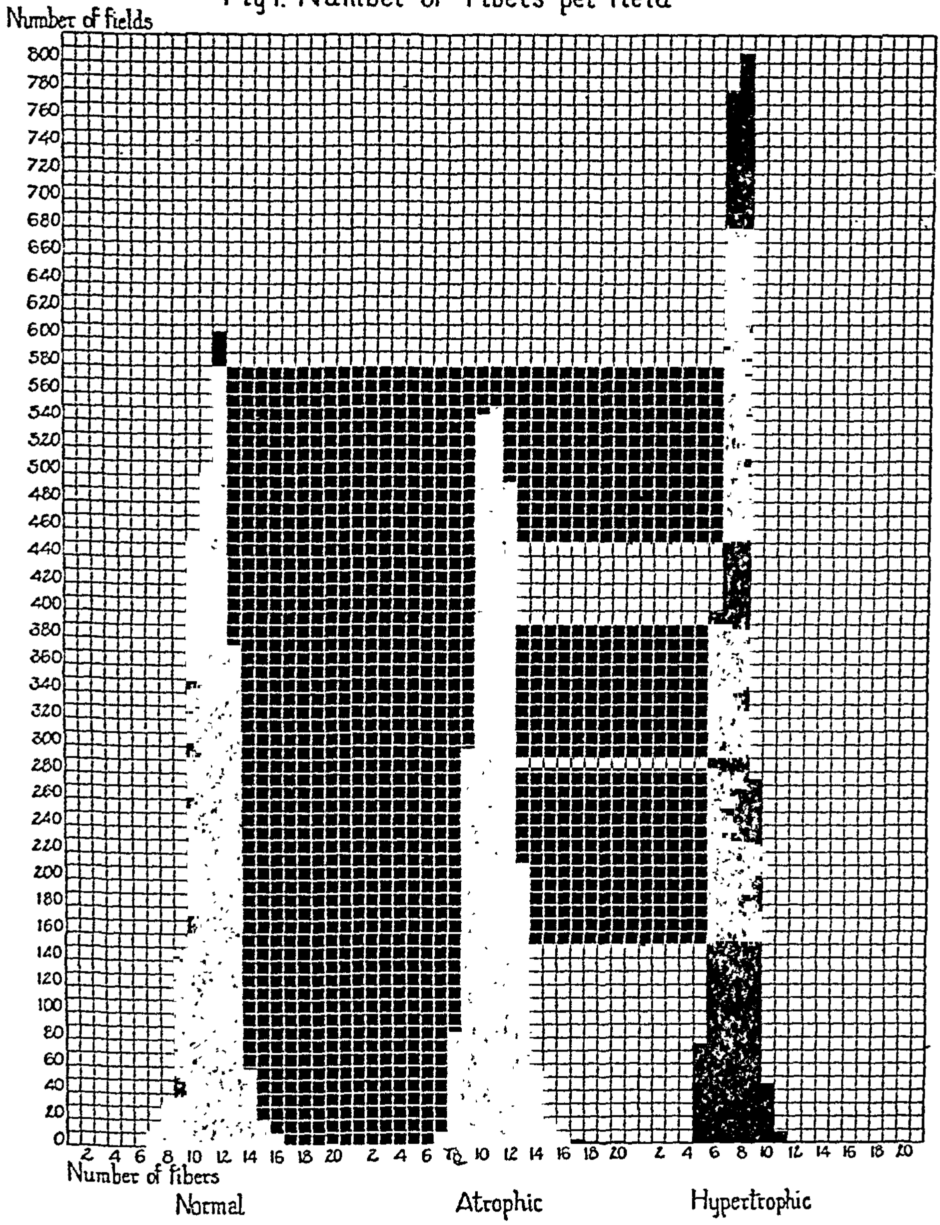
Number of fibers	Number of fields		
	Normal	Atrophic	Hypertrophic
5	0	0	76
6	0	0	398
7	10	8	778
8	31	86	808
9	149	298	266
10	451	546	42
11	502	548	8
12	612	494	0
13	376	212	0
14	59	60	0
15	19	16	0
16	9	4	0
17	2	2	0
18	2	0	0
19	2	0	0
	2,224	2,274	2,376

the larger numbers of fibers. The small distribution of fields indicates an approach toward greater uniformity than is true of the normal heart. In the atrophic heart the curve differs so that although the maximum of fields is in those which contain 11 fibers (average 10.8), nevertheless, the top of the curve is more flat than in the normal. It rises to and falls from its summit more steeply than the curve of the normal heart and presents fewer fields containing more than 11 fibers. These facts point toward a disposition to greater uniformity than is true of the normal.

The probable error of an average is a figure which can be predicted by the aid of Karl Pearson's tables. It naturally depends upon the size of the sample and the variation about the mean. Pearl defines it as a conventional measure of the reliability of re-

sults or of their "scatter" due to the chance effects of sampling. It is a constant so chosen that when its value is added to and subtracted from the average obtained for a particular sample the

Fig I. Number of Fibers per field



chances are even that the true average lies on the one hand within the limits thus set by the probable error or, on the other hand, between these limits and infinity in either direction. Thus, we find from a study of the normal heart that the average number of fibers

per field is 11.5 ± 0.021. Then the mathematical probability that the true average for *all* the fibers of this heart of which we have studied a sample lies between 11.479 and 11.521, is exactly equal to the mathematical probability that the true average falls outside these limits. The significance of calculations upon this basis is furnished by the discussion of results of this study.

The breadth of the fibers
(in arbitrary units)

	Maximum	Minimum	Average
Normal heart	0.9	0.3	0.503 ± 0.0015
Atrophic heart	0.7	0.3	0.438 ± 0.0010
Hypertrophic heart	1.15	0.45	0.907 ± 0.0023

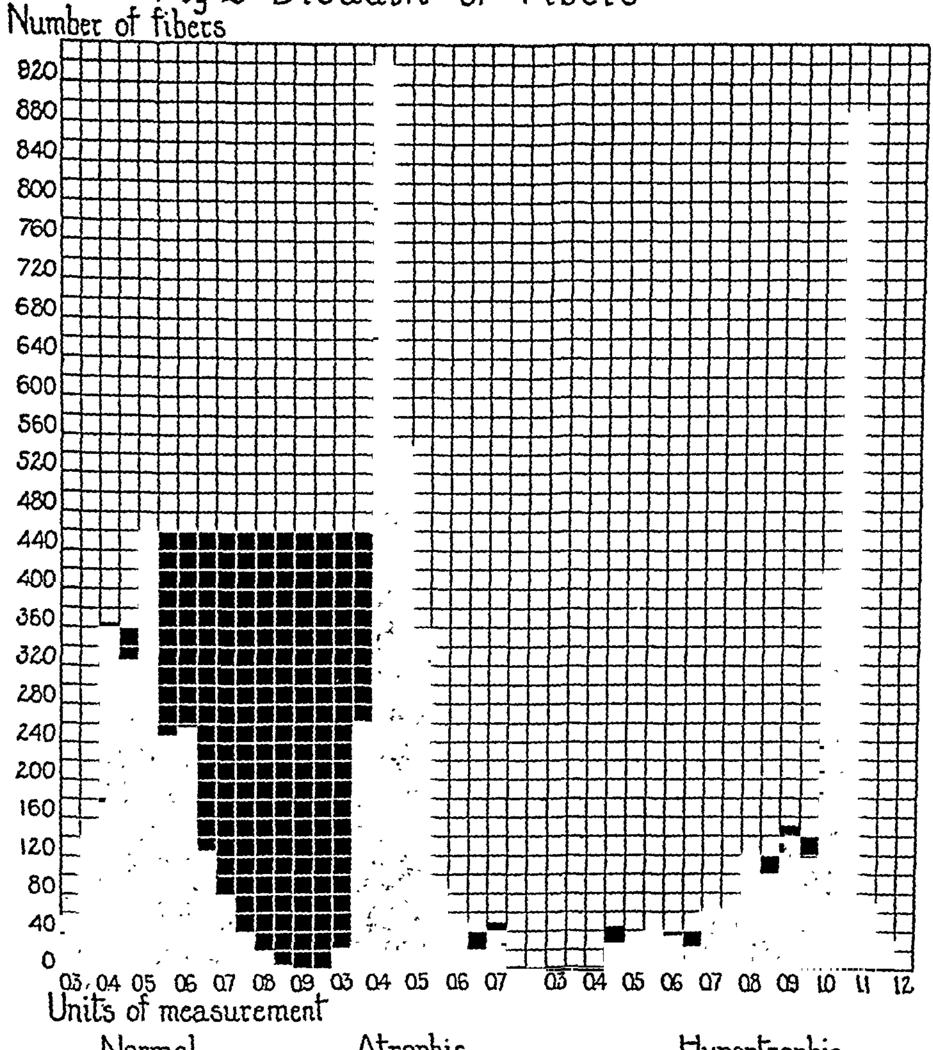
The number of measurements is so large, namely more than 2000 in each heart, that the error of random sampling is small and the results could not be significantly affected by further measurements, within reasonable limits. The measurements show differences in the

TABLE 2
The breadth of the fibers

Breadth of fibers (arbitrary units)	Number of fibers		
	Normal	Atrophic	Hypertrophic
0.3	54	22	0
0.35	142	264	0
0.4	372	940	0
0.45	328	546	46
0.5	467	348	28
0.55	251	86	38
0.6	258	52	36
0.65	128	20	26
0.7	82	52	60
0.75	45	0	62
0.8	20	0	124
0.85	5	0	102
0.9	1	0	156
0.95	0	0	110
1.0	0	0	418
1.05	0	0	890
1.10	0	0	72
1.15	0	0	38
	2,153	2,330	2,206

three hearts, with the same significance of results as exhibited by the number of fibers per field. An analysis of the figures is given in Table 2 and graphically represented in Fig. 2. In the normal heart the greatest number of fibers approximate in breadth the average. In the atrophic heart the greatest incidence is slightly to

Fig 2 Breadth of Fibers



the left of the average and in the hypertrophic heart distinctly to the right. As compared with the normal heart, the hypertrophic heart shows a much greater incidence of large fibers as well as many fibers larger than those in the normal. Conversely the atrophic heart shows a greater incidence of small fibers, but none smaller

than those observed in the normal (see Table 2). Since longitudinal splitting is said to occur in atrophic heart muscle it is possible that the increase in number of small fibers is due to this phenomenon, but if this were true there should be a considerable number of fibers smaller than those in the normal. No fibers of less than 0.3 unit (10.5 micra) were observed in either the normal or the atrophic heart. The number with this diameter is slightly smaller than in the normal heart, which suggests that in atrophy the fibers which were originally small undergo complete atrophy. By this method, as well as by calculating the number of fibers per field, the changes indicate an approach to uniformity in the phenomena of hypertrophy and atrophy.

Number of nuclei per field

	Maximum	Minimum	Average
Normal heart	26	5	11.0 ± 0.047
Atrophic heart	39	8	22.7 ± 0.075
Hypertrophic heart	20	5	9.8 ± 0.032

As would be expected the number of nuclei per field in the hypertrophic heart is less than in the normal heart. The number of nuclei per field in the atrophic heart is increased, but, as will be shown, in greater proportion than would be expected from the number of fibers per field and the breadth of the fibers. The figures are analyzed in Table 3 and shown graphically in Fig. 3. The number of fields containing the largest number of nuclei is to the left of the mean in the normal and hypertrophic hearts, and to the right in the atrophic heart. It is obvious, however, that in the hypertrophic heart fields containing a reduced number of nuclei predominate, whereas in the atrophic heart fields containing large numbers of nuclei are vastly in the majority.

The immediate problem is the question as to whether the increase in number of nuclei is due to crowding together or an actual multiplication. The most careful search has failed to disclose mitotic figures. A study of 29 atrophic hearts has shown no mitoses. It is, of course, possible that since the hearts are obtained post-mortem any mitotic figures present might have been completed. Subdivision of nuclei in various forms is present as shown in the photomicrographs, taken from the heart under special study. Similar pictures were found in 25 of the 29 other atrophic hearts examined. They may also occur in degenerations and in various forms of myo-

TABLE 3

Number of nuclei per field

Number of nuclei	Number of fields		
	Normal	Atrophic	Hypertrophic
5	3	0	18
6	30	0	110
7	116	0	288
8	370	6	466
9	292	6	392
10	350	0	416
11	183	6	290
12	247	8	220
13	158	46	200
14	103	58	70
15	100	106	74
16	43	104	14
17	33	150	8
18	25	124	8
19	26	158	4
20	25	182	6
21	10	102	0
22	8	146	0
23	5	136	0
24	3	152	0
25	4	174	0
26	4	132	0
27	0	110	0
28	0	98	0
29	0	94	0
30	0	136	0
31	0	10	0
32	0	72	0
33	0	18	0
34	0	22	0
35	0	44	0
36	0	10	0
37	0	6	0
38	0	8	0
39	0	9	0
	2,143	2,433	2,584

carditis. In lower forms amitotic division of cardiac muscle nuclei occurs (Conklin). We have observed mitotic figures in the fetal human heart but not in the adult nor in the dog heart. Herzog, how-

ever, claims to have observed both mitosis and budding in the heart muscle of victims of poisoning by illuminating gas. It is possible that these nuclear forms in the atrophic heart are of degenerative character, as observed by Van der Stricht and Todd in the conduction system, and the marked irregularity of the "corduroy" nuclei suggests that this is true. It might be thought that the vast increase in number of nuclei is due to counting the dumb-bell forms as two nuclei. Since the nuclei were counted with the 16 mm. objective an error of this kind is admittedly possible. Nevertheless, these forms constitute only about 2.5 per cent of the nuclei, which is of little significance in the 50 per cent increase observed.

For the purpose of demonstrating in simpler form the change in number of nuclei in relation to the fibers, a unit area may be assumed. The unit area is represented by the average number of fibers per field multiplied by the average breadth of fiber. The fiber unit area may be likened to a mat composed of fibers laid lengthwise side by side. Upon this mat the nuclei are scattered. The fiber unit areas for each heart are as follows:

Normal heart	5.75
Atrophic heart	4.75
Hypertrophic heart	6.64

By simple proportion, the number of nuclei in the hypertrophic and atrophic heart can be compared with the number in the normal heart. Assuming that there is no change in the distribution of nuclei upon the mat, the number of nuclei per fiber unit area should be 9.5 for the hypertrophic heart and 13.3 for the atrophic heart. The fiber unit area is for this purpose the effective field, since it disregards adventitious spaces. The observed average of nuclei per field in the hypertrophic heart is 9.8. This value is so close to the calculated value that it appears to demonstrate absence of change in relative number of nuclei when hypertrophy occurs. The observed number of nuclei per field in the atrophic heart is 22.7. Since the calculated value is only 13.3, it is apparent that the number of nuclei in the atrophic heart has risen to 150 per cent of the normal. As will be seen subsequently, the total number of fibers in the atrophic heart is reduced about 50 per cent. It might, therefore, be assumed that the increase in number of nuclei is apparent rather than real and due to a crowding together of the nuclei as atrophy progresses.

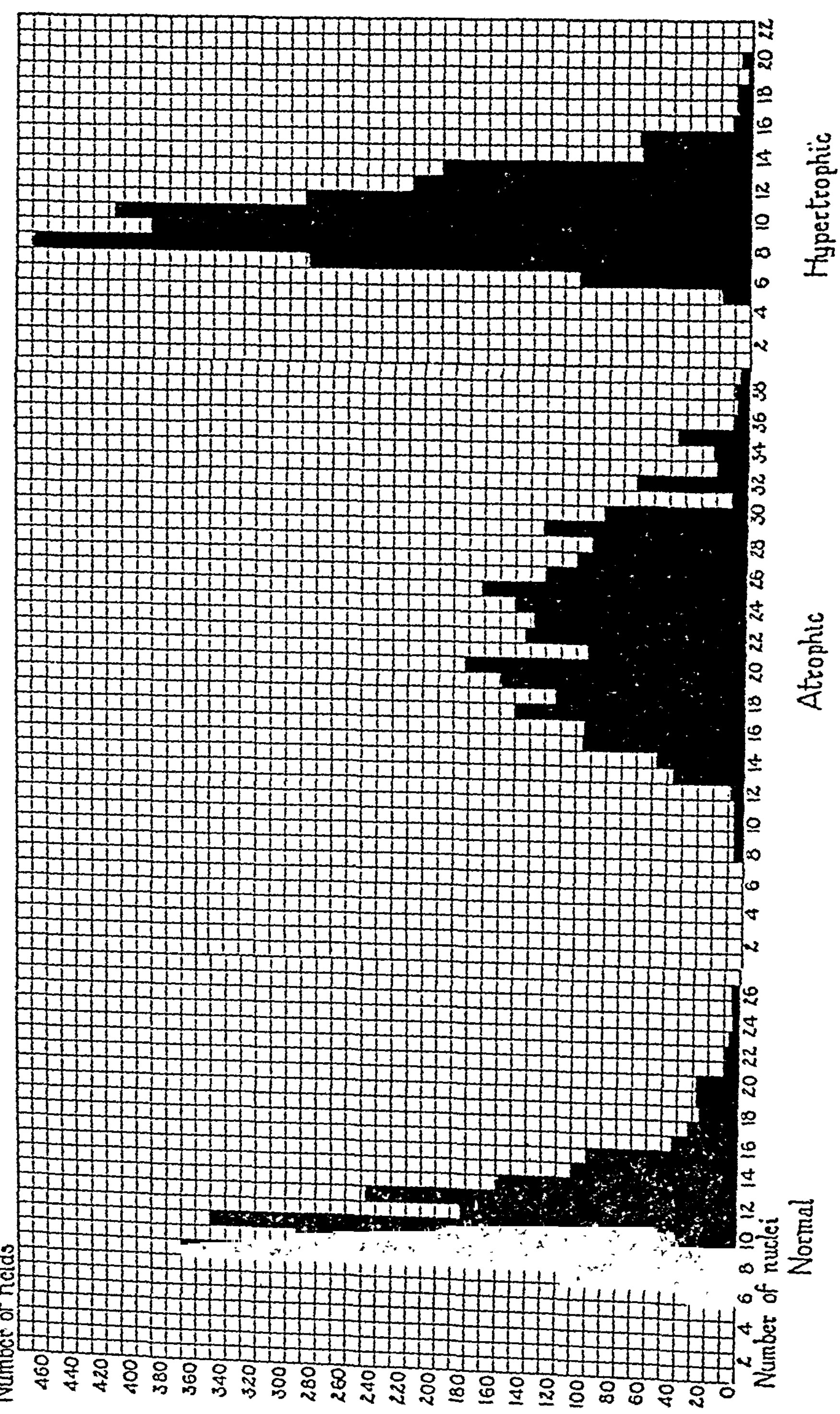


Fig. 3 Number of Nuclei per field

Since it is shown, however, that atrophy is accompanied by a loss of fibers this assumption would necessitate acceptance of the view that fibers may disappear and nuclei remain, which is not in accord with the generally accepted conception of cell form and nutrition. Furthermore, in the hearts studied all the nuclei counted were placed centrally in the fiber. The inference is plain that there must be in some way, either by mitosis or direct division, a multiplication of the muscle nuclei. This view has further support in the work of Edens, quoted above.

Discussion

The specific gravity of the normal and hypertrophic hearts is about the same, whilst that of the atrophic heart is slightly greater. Fibrosis is not a prominent feature in any of these hearts and if the alteration of specific gravity be significant, a condensation of solids must be assumed in the atrophic heart. With this difference of specific gravity and for other obvious reasons, it has seemed wise to attempt an estimation of total number of fibers upon the basis of volume rather than weight. The use of serial sections 5 micra thick makes it possible to calculate in terms of three dimensions. The figures arrived at are comparable but not to be regarded as absolute because of the syncytial character of heart muscle. The results are as follows:

Normal heart

Volume 295 c.c. Weight 300 gm.
Average 11.549 fibers in 0.00015 c.mm.
22,715,000,000 fibers in 295 c.c.
7,700,000,000 fibers in 100 c.c.

Atrophic heart

Volume 160 c.c. Weight 165 gm.
Average 10.801 fibers in 0.00015 c.mm.
11,520,000,000 fibers in 160 c.c.
7,200,000,000 fibers in 100 c.c.

Hypertrophic heart

Volume 485 c.c. Weight 500 gm.
Average 7.394 fibers in 0.00015 c.mm.
23,894,010,000 fibers in 485 c.c.
4,926,600,000 fibers in 100 c.c.

It is admitted that whatever error is present in the primary figures is multiplied in these estimates of the number of fibers in the hearts

or in the constant volume of 100 c.c. Nevertheless, it is probable that the total number of fibers in the hypertrophic heart is about the same as in the normal. In the atrophic heart, on the basis of this calculation and the others reported in the results of the study, the total number is reduced and this reduction is at the expense of the larger and smaller fibers. In the hypertrophic heart the individual fibers are large. Consequently, the number per 100 c.c. is reduced in proportion but the total number in the heart remains the same as in the normal. In the atrophic heart no fibers are found smaller than in the normal heart and the average fiber thickness is approximately that found in the normal heart. Hence, the number of fibers per 100 c.c. remains essentially the same as in the normal heart but the total number of fibers is reduced in general proportion to the smaller heart volume. It is suggested that there is a minimum to which reduction in size may go, beyond which the fiber rapidly disappears. The loss of transverse striation and vacuolization near the poles of the nuclei in the atrophic heart, as well as longitudinal splitting and reduced affinity for dyes, indicates a retrogressive change. This supports the studies of Bradley and his collaborators, who show definite autolysis in skeletal muscle. Perhaps the close relation of the number of fibers per constant volume to that of the normal heart is due to imbibition of water and the increased specific gravity due to a decrease in diffusibility of some of the products of disintegration.

Of further interest is the examination of variability. This is given in tabular form.

Variability of number of fibers per field

	Mean	Stand. Dev.	Coeff. of Var.
Normal heart	11.5 ± 0.021	1.45 ± 0.0146	12.61 ± 0.1294
Atrophic heart	10.8 ± 0.023	1.50 ± 0.0150	13.88 ± 0.1414
Hypertrophic heart	7.4 ± 0.015	1.07 ± 0.0104	14.46 ± 0.1441

This shows an essential uniformity of variability, from which it can be assumed that for all practical purposes the mean or average values really represent the number of fibers per field.

Variability of number of nuclei per field

	Mean	Stand. Dev.	Coeff. of Var.
Normal heart	11.0 ± 0.047	3.21 ± 0.0331	29.18 ± 0.3246
Atrophic heart	22.7 ± 0.075	5.65 ± 0.0546	24.89 ± 0.2551
Hypertrophic heart	9.8 ± 0.032	2.43 ± 0.0228	24.80 ± 0.2466

Variability of breadth of fibers
(in units)

	Mean	Stand. Dev.	Coeff. of Var.
Normal heart	0.503 ± 0.0015	0.102 ± 0.0010	20.404 ± 0.2186
Atrophic heart	0.438 ± 0.0010	0.072 ± 0.0071	16.409 ± 0.1690
Hypertrophic heart	0.907 ± 0.0023	0.160 ± 0.0016	17.550 ± 0.1826

In both these sets of figures it is found that the variability is greatest in the normal heart and definitely reduced in the hypertrophic and atrophic hearts. In other words both these changes tend toward greater uniformity. Variability is a characteristic of normality and constitutes Nature's provision for emergency. It seems to be fundamental to adaptability. In hypertrophy the response to extra demands for work appears to be in the production not only of large fibers but fibers with a greater degree of uniformity than exists in the normal heart. By reference to Table 2, this is seen to be due to an elimination of smaller fibers probably because they increase in size. In the particular heart studied 1528 fibers of the hypertrophic heart, or 69 per cent of the 2212 fibers measured, are larger than any observed in the normal heart. The number of nuclei per field is also more uniform.

In the atrophic heart the change is associated with retrogressive processes. This is represented by a reduction in the absolute number of larger fibers. In the normal heart, fibers with a diameter of 0.9 units (31.5 micra) are found, but the largest in the atrophic heart measure 0.7 units (24.5 micra). In the normal heart, 42 per cent of the fibers are less than the average of 0.5 units whereas in the atrophic heart 76 per cent are less than that figure. At the same time the coefficient of variability is reduced and this indicates a greater uniformity in size of fibers than in the normal heart.

The tendency toward uniformity as an adaptation to abnormal demands and conditions furnishes a new conception of the place of variability in adaptation.

The Study of Variation in Cardiac Muscle Fibers. In medical and biological studies the maximum and minimum values in addition to the average are stated in order to give some idea of the variability of the sample. The defect of this method is the apparent accidental nature of the precise maximum and minimum values noted. Further, these figures give no indication of the type of "scatter" about the mean value. In modern studies it is customary to replace the

maximum and minimum by a constant, known as the standard deviation. This is a quantity borrowed from theoretical mechanics in which it is known as the radius of gyration. For the purpose of this paper it is sufficient to state that within the limits of the standard deviation from the mean lie one half of all the individual units measured; in practice more than half may be found within these limits. None but "stragglers" lie beyond the limits defined by three times the standard deviation. Thus, in Table 1, the stragglers number six, eight and six in normal, hypertrophic and atrophic hearts respectively.

So long as it is desired to compare variability of a single character in several samples the standard deviation gives a convenient measure provided the mean values are approximately the same. Thus, the mean numbers of fibers per field in the normal and atrophic hearts are 11.5 and 10.8 respectively and we find these standard deviations are 1.45 and 1.50. But as soon as an attempt is made to compare variabilities in the same character when the means are widely divergent or when comparison is made of different characters the standard deviation fails to express clearly what the difference may be. Hence, a new value is calculated by ascertaining the percentage on the mean of its own standard deviation. This measure is the coefficient of variation. In the sample just quoted the standard deviations are respectively 1.45 and 1.50; the corresponding coefficients of variation are 12.61 and 13.88. When number of fibers per field is compared in the normal and hypertrophic hearts the means are quite divergent, namely 11.5 and 7.4. Their standard deviations, 1.45 and 1.07, do not readily disclose the precise difference in variation. Reference to the coefficients of variation defines this difference at once; it is represented by the figures 12.61 and 14.46.

Suppose one undertake, in the normal heart, to compare variability in number of fibers per field with number of nuclei per field. These are clearly different characters and the standard deviations, 1.45 and 3.21, are not directly comparable. But the coefficients of variation, calculated upon standard deviation and mean, at once make this comparison possible. Variability in number of fibers is 12.61 per cent and variability in number of nuclei is 29.18 per cent. It must not be assumed, however, that variability in nuclei is twice that of variability in fibers for, by the method of this investiga-

tion, fibers are counted in one dimension only of the field whereas *nuclei* are counted in two dimensions. In all such comparisons reservations must be made for the number of dimensions involved in the treatment of the character. This reservation can be mathematically computed (Miner). Comparing, in the normal heart, number of fibers per field with breadth of fiber one obtains the coefficients of variation 12.61 and 20.404 respectively. It is legitimate to assume that variability in fiber breadth is almost double that in number of fibers per field since both values involve merely a single dimension.

It is naturally desirable to have some absolute standard by which these variabilities may be judged. The simplest single-dimensional standard is the standing height of the body. The coefficient of variation for standing height in the male white dissecting room population has been ascertained to be 3.55 per cent (Todd). That a variability of 20.4 per cent should be obtained for breadth of normal cardiac fibers is by no means surprising, for the whole is less variable than its component parts. Indeed the restraint of variability in so minute a dimension is especially noteworthy.

Weight of the heart is closely related to volume and hence may be considered a three-dimensional character. The weight of the "normal" heart is given by Greenwood as having a coefficient of variation 17.71 per cent, that of the diseased heart has a variability of 32.39 per cent (Greenwood). The "normal" hearts are those which showed no evidence of disease at autopsy; the diseased heart series contained a large number of cases of valvular disorder as well as atrophic organs and this accounts for the relatively large variability. The three-dimensional variability of 17 per cent corresponds in practice to a single dimensional variability of about 8 per cent, and upon this standard the several variabilities recorded in this paper may be judged.

Comparison of Mean Values. To the mind unaccustomed to statistical treatment it may appear that the difference between some of mean values here recorded is so small that it should not be emphasized. But mathematically, the real value of the divergence may be gauged upon the theory of error. As an example, the difference between the mean values of number of fibers per field in the normal and atrophic hearts may be taken. The difference between the mean values is 0.7 (i. e., 11.5–10.8). The probable error of these averages depends upon the number in the sample and the variation in the

sample. Mathematically, it can be shown that the probable error of the difference between these averages is given by the square root of the sum of the squares of the probable errors of the two averages. One can, therefore, compute the relation of the difference between the averages to its probable error, or better to the standard deviation of the difference. By a simple technique this can be referred to the normal curve of probability, or, as it is often called, the Gauss-Laplace curve of error. Thus, one may calculate the chances that, in random sampling, the difference between the average values may or may not be reached or exceeded. In the example chosen the improbability of bridging this difference is represented by the figures $\frac{3.671}{10^{50}}$. This corresponds to odds so great that the certainty of a real difference in mean values is established beyond possibility of doubt.

Table 4 gives the result of an enquiry into the validity of the differences found between the mean values in all the previous tables. The odds in favor of a real difference between the mean values is so overwhelming that the validity of the difference cannot be disputed. Mathematically, it is found that a difference which is four times its probable error will arise less than once in one hundred trials. The odds in favor of a real difference between mean values, when this difference reaches eight times its probable error, are beyond any human power of conception. The results in Table 4 are given in terms of the standard deviation of the difference and this equals the probable error divided by the constant .6745. It is therefore about one and a half times the probable error. Hence, if the difference exceeds eight times its standard deviation the improbability of confusion in mean values is still more striking. To render more apparent the difference between mean values quoted in this paper there is added to Table 4 a statement of the ratio: difference between means divided by standard deviation. Comparison of these figures with the index of improbability indicates how rapidly the odds mount after the ratio difference divided by standard deviation reaches 3.

In this statistical survey it must be realized that mathematical presentation is merely a yardstick for measuring differences. Nothing can be obtained by mathematical treatment which was not there in the beginning. And some methods of treatment fail to bring out

the really significant facts. For example, Table 4 demonstrates that there is a real difference between the number of fibers per field in the normal and atrophic hearts, and again between the number of nuclei per field in the normal and hypertrophic hearts. It, of course, implies no more than this. Both these cases have been elucidated earlier in the paper. In the former case it has been shown that

TABLE 4

Improbability of confusion of mean values

	Improbability figure	$\frac{\text{Diff.}}{\text{Stand. Dev.}}$
Number of fibers per field		
Normal and atrophic hearts	$\frac{3.671}{10^{50}}$	15.2
Normal and hypertrophic hearts	$\frac{1.946}{10^{5322}}$	107.9
Number of nuclei per field		
Normal and atrophic hearts	$\frac{8.243}{10^{2051}}$	96.7
Normal and hypertrophic hearts	$\frac{7.794}{10^{45}}$	14.29
Breadth of fiber		
Normal and atrophic hearts	$\frac{2.781}{10^{127}}$	24.1
Normal and hypertrophic hearts	$\frac{1.344}{10^{2174}}$	100.0

the significant difference is the more uniform number of fibers per field (flatter curve) in the atrophic heart. In the latter case the genuine difference in number of nuclei can be discounted by reference to the fiber unit area.

SUMMARY

The enlargement of the heart in hypertrophy is due principally to a hypertrophy of the muscle fibers without an increase in the number of fibers. There is then no hyperplasia of the fibers and the process is one of pure hypertrophy. The change is accompanied by a distinct tendency toward uniformity in breadth of the fibers. The average breadth is significantly greater than in the normal heart. It would appear that all fibers tend to approximate the larger size and it is suggested that when all or nearly all the fibers have attained the maximum, further hypertrophy is impossible. What

determines the maximum is not disclosed by this study, but the anatomical basis for a "limit of hypertrophy" can be explained on this basis. Presumably, also, the relative reduction of reserve capacity in the hypertrophic heart may be due to the fact that the individual fibers have reached their maximum growth and probably also their maximum functional strength.

The reduction in size of the heart in atrophy is due to a reduction in size of the muscle elements, with a decrease in the number of fibers of the whole heart. The fibers are more uniform in breadth than in the normal heart. Since there are no fibers whose breadth is less than the narrowest fibers of the normal heart, the change must be due to an atrophic shrinkage and not to longitudinal splitting. The approach to uniformity is associated with a disappearance of the larger fibers, which have decreased in breadth toward the smaller average. Furthermore, many fibers have disappeared completely since the number of fibers in the entire heart is significantly decreased. All these changes are concordant with the conception of autolysis in atrophy as demonstrated by Bradley.

In proportion to the number of fibers the number of nuclei in the atrophic heart is vastly increased. This is clearly shown by mathematical calculations and is in accord with Edens's demonstration of relative increase of purine nitrogen in the atrophic heart. This might be regarded as an attempt at regeneration but no mitotic figures or clear indications of amitotic division were found. The increased number, however, is well established and must be due to some form of multiplication, but this study gives no information as to the fiber units (or cells) and does not correlate nuclei and fiber units. To elucidate the increased number of nuclei further work is necessary to demonstrate clearly and uniformly the intercalated discs.

The approach to uniformity in breadth of the fibers of the hypertrophic and atrophic heart as compared with the normal gives a new conception of variability as affecting the cardiac muscle in its adaptation to abnormal conditions.

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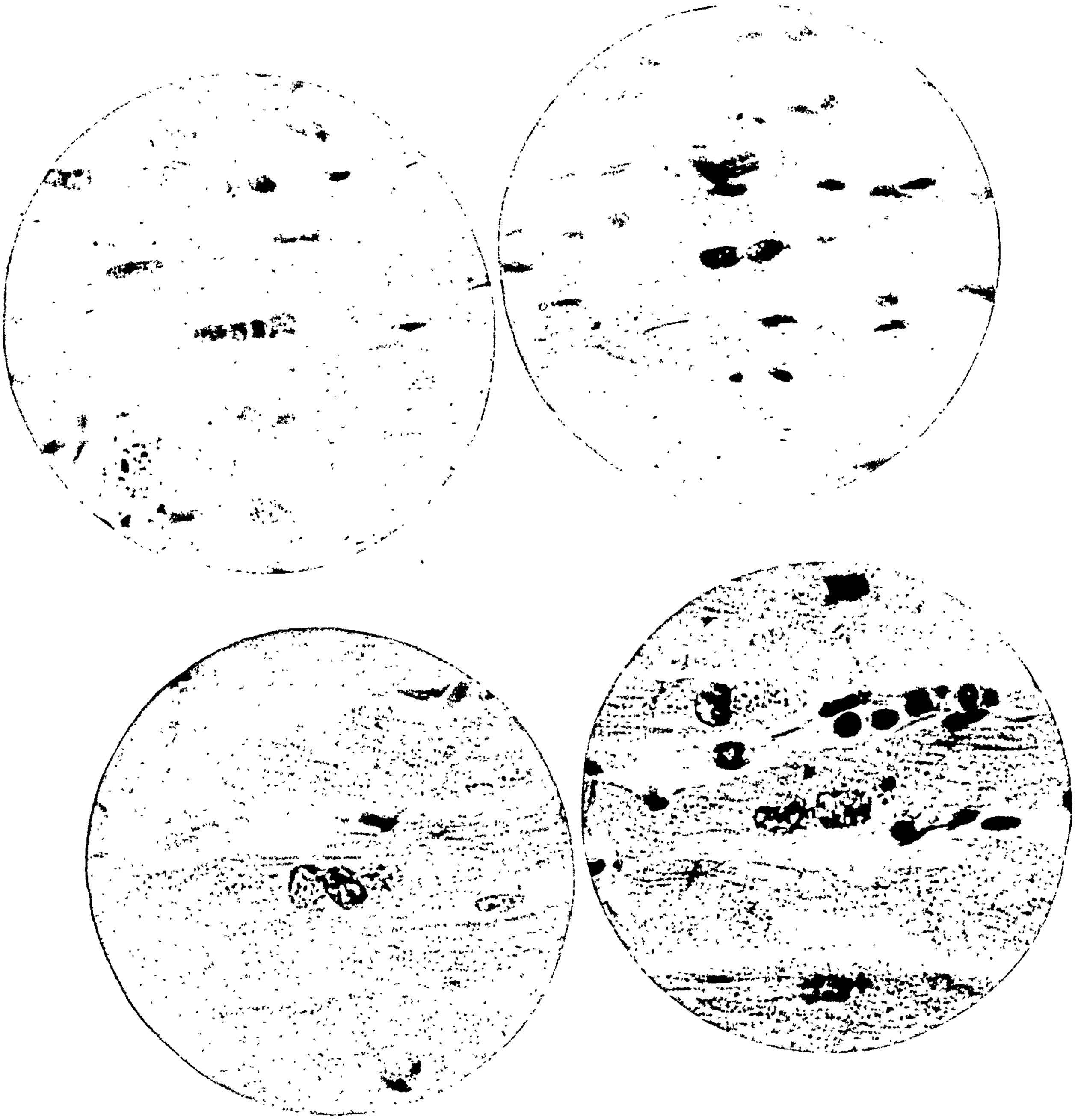
DESCRIPTION OF PLATE LVIII

Photomicrographs (4 mm. obj.) of various forms of nuclei in the atrophic heart. Note the "dumb-bell" and "corduroy" forms.



Karsner, Saphir and Todd

The cardiac muscle in hypertrophy and atrophy

THE HISTOLOGICAL ALTERATIONS OF THE PANCREAS IN CHRONIC PASSIVE CONGESTION *

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The purpose of this paper is to call attention to certain striking changes in the pancreas found in chronic venous congestion. The lesions associated with venous stasis in the lungs, liver, spleen and kidneys have separately been described; those in the pancreas are dismissed with the casual statement that they occur. We have found no detailed and minute description of these changes in the pancreas, either in the better known text-books or in the available periodical literature. To show how little attention has been paid to these lesions, the following references may be cited:

"Passive hyperaemia (of the pancreas) occurs in conditions of general passive congestion." (Adami and Nicholls.) [1]

"Longstanding difficulty of venous flow, due to chronic disease of the heart, liver, lungs, etc., may cause induration and fibrosis of the pancreas, as of other organs." (Mayo Robson and Cammidge.) [2]

"Among the secondary diseases of the pancreas must be mentioned metastatic tumors and abscesses; also hyperaemia and chronic interstitial inflammation, the result of venous congestion in chronic affections of the heart, lungs, or liver." "Passive hyperaemia may result from venous congestions, in consequence of heart, lung or liver disease; in such cases often combined with evidence of interstitial connective tissue, hyperplasia, and consequent increase of volume and consistence of the organ." (Friedreich.) [3]

"The frequency of chronic congestion of the abdominal viscera, and the relative infrequency of chronic interstitial inflammation of the pancreas, is evidence that the former condition is not commonly a cause of the latter. Chronic passive congestion may doubtless produce slight proliferation of the interstitial connective tissue, but is an unimportant factor in the production of chronic pancreatitis." (Opie.) [4]

* Received for publication April 12, 1925.

Our attention had for some time been attracted by atrophic changes in the pancreatic parenchyma occurring in cases of uncompensated cardiac disease with general venous stasis. In order to study this condition more systematically, and to establish, if possible, a precise relationship of these pancreatic changes to venous stasis, we have studied sections of the pancreas from 100 cases showing by histological examination chronic passive congestion of the liver, more or less intense. The pancreas in these cases was examined without reference to the clinical data. As control material 100 cases were selected in which there was neither clinical nor pathological evidence of long-standing impairment of the circulation. They were chosen from individuals of comparable ages.

In many instances only one section of the pancreas was available for study; in other cases there were sections from the head, middle and tail portions of the organ. The sections were examined with various stains: hematoxylin and eosin; Van Gieson's picro-fuchsin; Mallory's aniline blue-orange G and Heidenhain's iron hematoxylin.

Nature of the Lesions

Before analyzing this material in detail, the alterations which have seemed to us distinctive of chronic passive congestion of the pancreas may be described. The most striking feature, apparent at a glance, is an atrophy involving portions of each lobule (Fig. 1). While this atrophy does not affect the lobule uniformly, each lobule is to a greater or lesser degree involved; and in general the peripheral portion more than the central. It may be seen further that the islands of Langerhans are invariably surrounded by a zone of normal pancreatic tissue; and this is especially distinct in the most advanced cases in which only those acini in immediate contact with the islands show normal structure and dimensions (Fig. 2).

In contrast with the normal acini, in which the epithelial cells are ranged about a central lumen, and contain the normal prezymogen granules, the atrophic acini frequently show no lumen; the cells are reduced in size, their nuclei closely aggregated and shrunken (Fig. 3). The specific granules have disappeared.

There is no evidence that this atrophy leads to a true fibrosis of the organ as in some other tissues; the shrinkage of the parenchyma is followed or accompanied by a condensation of the connective tissue stroma and the basement membrane is not swollen. Very rarely

are a few small mononuclear wandering cells found in the atrophic areas. As Opie has already pointed out, there is no justification for the view of Friedreich and of Mayo Robson and Cammidge that chronic venous congestion may lead to fibrosis or chronic pancreatitis.

A point which must be discussed in this connection is the possibility that shrinkage during fixation may be confused with atrophy. It is impossible to avoid this artifact in routine post-mortem material no matter how careful the technique. There are, however, obvious differences between the artificial shrinkage and the tissue atrophy associated with venous stasis. In the former case, the cells are retracted from the basement membrane; in the latter, there is no retraction, the basement membrane shrinking with the acinus. Furthermore, the association of the atrophy with congestion of the capillaries and veins, and the more or less typical distribution of the atrophic areas, serve to eliminate the artificial effect produced during fixation. Finally, fixation shrinkage must have occurred with equal frequency in the control material, and yet as the tabulated observations show, none of these cases exhibited changes comparable to those found in chronic passive congestion.

Relation of the Lesion to Cardiac Decompensation

The cases comprising this series are readily divided into two groups: the first, those cases of long-continued or oft-repeated passive congestion associated with primary cardiac disease; and the second, cases of decompensation subsequent to diseases other than primary cardiac disease (Table 1).

In the first group are 72 cases and it will be noted that of these, 57 showed alterations in the pancreas.

Of the 28 cases in the second group, only 7 showed any changes in the pancreas which could be related to the passive congestion.

1. *Cases of primary cardiac disease with decompensation.* Further analysis of the histories of the 72 cases in this group was made to determine if there existed any relation between the time which elapsed after the first break in cardiac compensation and the degree of damage to the pancreas. Such study showed that there was no constant relation between the two, even though there may have been several periods of decompensation in the interval between the first break and the death of the individual.

More closely correlated with the severity of the lesion is the duration of the final period of cardiac failure. It was found indeed that

TABLE 1

		Atrophic changes in the pancreas			
		Slight	Moderate	Advanced	Negative
Cases of primary cardiac disease with decompensation	72	15	20	22	15
Cases of decompensation subsequent to diseases other than primary cardiac disease	28	4	2	1	21
Control cases	100	—	—	—	100

the extent of the damage to the pancreas is directly proportional to the length of the last break in the compensation of the heart. By reference to Table 2, it will be noted that no demonstrable change could be detected in the pancreas in those cases with an average

TABLE 2

Number of cases	Average duration of last period of decompensation	Changes in pancreas
15	10 days	0
15	33 days	slight
20	43 days	moderate
22	93 days	advanced

duration of 10 days; slight changes were present in those cases of 33 days; moderate, in 43 days, while in the cases in which the atrophy was advanced, the final period of decompensation was of a duration averaging 93 days.

In general it is found also that the alterations in the pancreas follow closely those existing in the liver. The more advanced the lesions of chronic passive congestion in the liver, the more intense were those in the pancreas (Table 3). There were, however, exceptions

to this finding, namely those cases in which the final period of decompensation was of short duration, and in these cases the altera-

TABLE 3

Chronic Passive Congestion of the Pancreas and Liver
Comparison of the Intensity of Alterations

Pancreas		Liver		
		Slight	Moderate	Advanced
Negative	15	4	6	5
Slight	15	1	4	10
Moderate	20	4	5	11
Advanced	22	0	7	15

tions in the liver were advanced in some instances while the pancreas showed no changes, or the alterations were slight (Table 2).

2. *Cases of decompensation subsequent to diseases other than primary cardiac disease.* Included in this group are a variety of diseases, such as chronic nephritis, etc.

The number of the cases showing alterations in the pancreas is too small to permit any conclusions to be made.

The question naturally arises whether it is possible to explain the distribution of the areas of congestion and atrophy in the pancreas on an anatomical basis. The study of the structure of the pancreatic lobule is necessary to answer this query.

In the human pancreas the distinct divisions as seen in the gross specimens are usually designated as lobules. These lobules are not individual units but are in reality made up of smaller masses of tissue which are described as the primary lobules. These primary lobules, not clearly separated from one another, are further united into groups varying much in size. Such lobule groups, or secondary lobules, are separated from each other by bands of loose connective tissue in which course the nerves, vessels and larger ducts. The secondary lobules are readily seen in the gross specimen and are grouped together to form the largest subdivisions or tertiary lobules. For the designation of these largest divisions some prefer the term

lobe of the pancreas. The islands of Langerhans in the human pancreas do not occupy any seemingly definite position in the primary lobules, though they are often situated near the centre.

The blood vessels accompany the ducts until the secondary lobule is reached. The arteries then no longer lie parallel to the ducts. In injected specimens a large artery can be seen penetrating to the central part of the secondary lobule where it divides rapidly and small branches ramify through the primary lobules. The veins run parallel to the arteries. Histologically it has seemed to us that the differentiation of arterioles from the smaller veins is one of great difficulty.

The circulation of the islands is of interest and importance with reference to the changes seen in chronic passive congestion. Many observers find a definite glomerular network in the islands. This network is made up of freely anastomosing channels much larger than capillaries and from these island vessels many capillaries pass between the acini immediately adjacent to the island. The nature of these vessels within the islands has been the cause of considerable discussion. von Ebner,[5] and De Witt[10] believe them to be venous; Kühne and Lea,[6] and Opie[7] think that they are closely connected with arterioles, though there is no definite afferent vessel to be made out. Opie,[7] in the pancreas of the cat, using granular injection masses through the arteries, found that the island vessels were filled and the interacinar capillaries contained little of the injected material; also, when a non-granular injection mass, such as Berlin blue, was used and the injection incomplete, that the islands often were injected and the nearby capillaries were for the most part empty. From these data he concluded that the glomerular network of the islands is in very free communication with the smallest arteries, and the blood supply to them is richer than it is in other parts of the lobule. Laguesse[8] states that generally a principal vessel enters the island, rarely an arteriole, generally a large branch continuous with an arteriole. Jordan and Ferguson[9] remark that "certain arterial branches also enter the islets and form a specially rich plexus of broad capillaries (sinusoids) within these cell groups."

De Witt,[10] working with the pancreas from many different animals as well as the human, came to the conclusion after double injections that the vessels in the islands were of venous origin, supporting the belief of von Ebner and others. Furthermore, in studying the pancreas from cases of chronic passive congestion, she found that there

was stasis of blood in the island capillaries and cites this in support of the venous origin of these vessels.

The areas of chronic passive congestion of the pancreas have been noted by us as being frequently at the periphery of the secondary lobule, the zone of congestion often extending toward the interior of the lobule. Small areas of congestion and atrophy occur also at various parts of the secondary lobule, being apparently quite irregular in distribution.

In reality, the position of these areas is not so irregular as would seem to be the case at first glance. When the changes are studied, beginning with those of slight degree, through the moderate to the advanced lesions, it becomes increasingly evident that these areas are found at the periphery of circulatory units. These circulatory units would appear to coincide with the primary lobules, the boundaries of which are sharply defined in the advanced cases by the ring of congested capillaries and atrophic parenchyma, with the island of Langerhans, surrounded by at least one row of acini with well-preserved cells, in the more central part (Fig. 2). This definition into lobules is best seen in the caudal portion of the organ, where the islands are most numerous.

The sinusoids of the islands of Langerhans are for the most part quite empty and we are unable to confirm the observation made by De Witt studying cases of chronic passive congestion. An occasional island is found with greatly engorged vessels or even hemorrhage but this ectasia occurs with equal frequency in the control cases and we do not consider it as distinctive of venous stasis. The engorgement of the capillaries becomes more marked as one passes outward from the islands, and this is especially well seen in the cases of advanced passive congestion (Fig. 2). Such findings would not be expected if the island vessels were of venous origin.

It is difficult also to escape the conclusion that the islands are closer to the arterial circulation and have a richer blood supply than the periphery of the primary lobule, for no changes have been detected in the island cells. Likewise those acini immediately adjacent to the islands escape injury because of their nearness to this better blood supply and their rich capillary anastomoses with the island vessels.

The distribution of the zone of congestion in the pancreas is quite comparable to that in the liver in severe chronic passive congestion,

the island of Langerhans occupying a position analogous to that of the portal area.

Summary

A comparative histological study of the pancreas has been made in 100 cases of cardiac decompensation and an equal number of cases in which there was no clinical or pathological evidence of chronic venous stasis.

In a large percentage of the cases of chronic passive congestion certain lesions are to be found in the pancreas, which are not present in the control series.

The distinctive features of chronic passive congestion of the pancreas are: (1) areas of capillary congestion at the periphery of the primary lobules, (2) atrophy of the parenchymal cells in the congested areas, (3) condensation of the connective tissue framework following or accompanying the atrophy of the cells, (4) disappearance of the prezymogen granules in the atrophied cells. It is also characteristic of this condition that there is no congestion of the vessels of the islands of Langerhans, no demonstrable change in the island cells, and no fibrosis in the congested portions.

The intensity of these changes in the pancreas follows closely that in the liver in chronic passive congestion, and is directly related to the duration of the final period of cardiac decompensation.

There is no evidence to support the belief that chronic interstitial pancreatitis follows chronic passive congestion of the pancreas.

We are indebted to Mr. Alfred Feinberg for the drawings.

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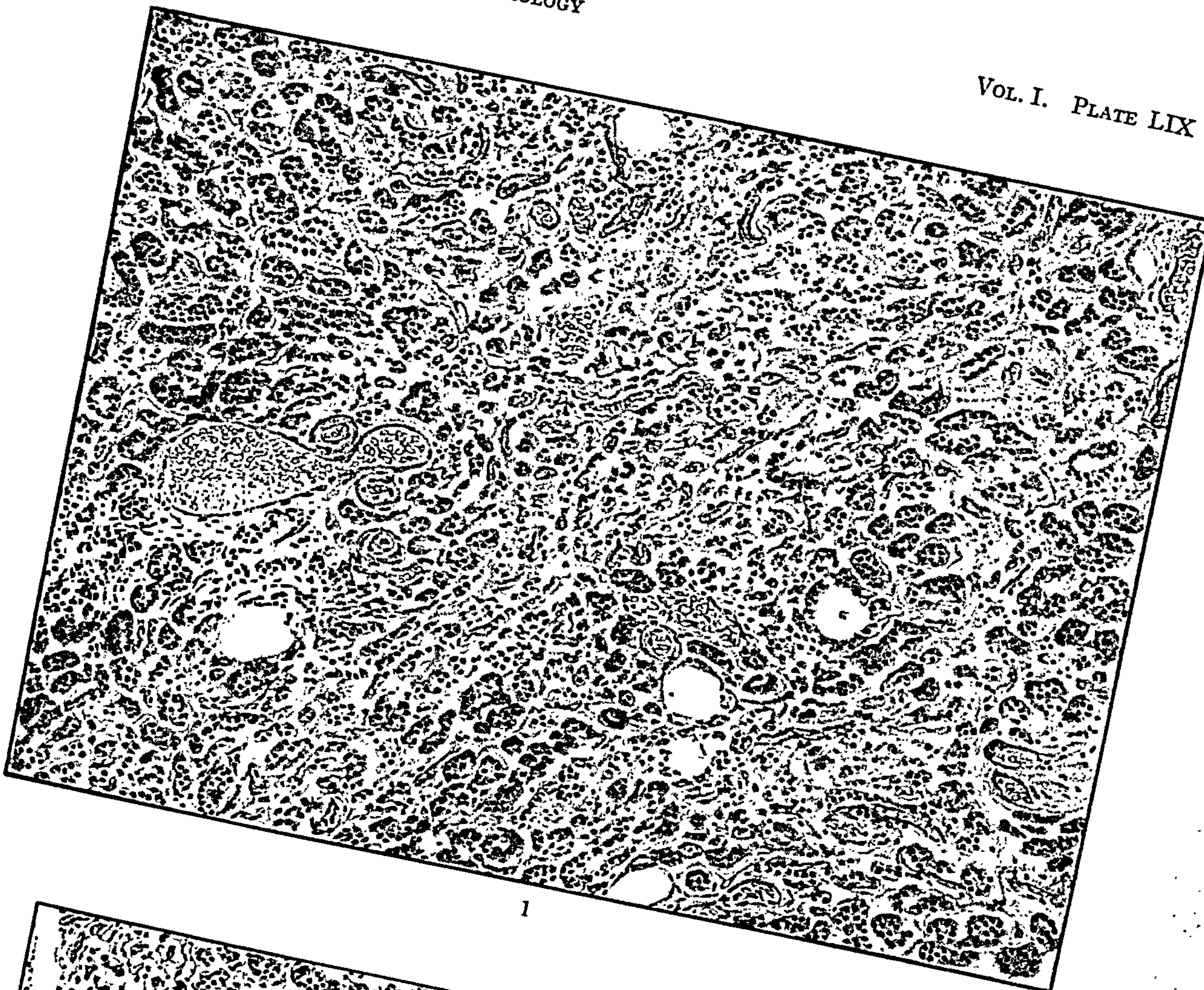
DESCRIPTION OF PLATES LIX–LX

Fig. 1. Pancreas. Chronic passive congestion of moderate intensity. Atrophy of the parenchyma in the area of congestion.

Fig. 2. Pancreas. Extreme chronic passive congestion. Parenchymal cells well preserved about the islands of Langerhans. Marked atrophy of the cells in the peripheral portions of the lobules.

Fig. 3. Chronic passive congestion of the pancreas. A: Normal cells in an area where there is no congestion. B: Atrophy of cells in a congested area. (Photomicrographs from the same section.) x 450.



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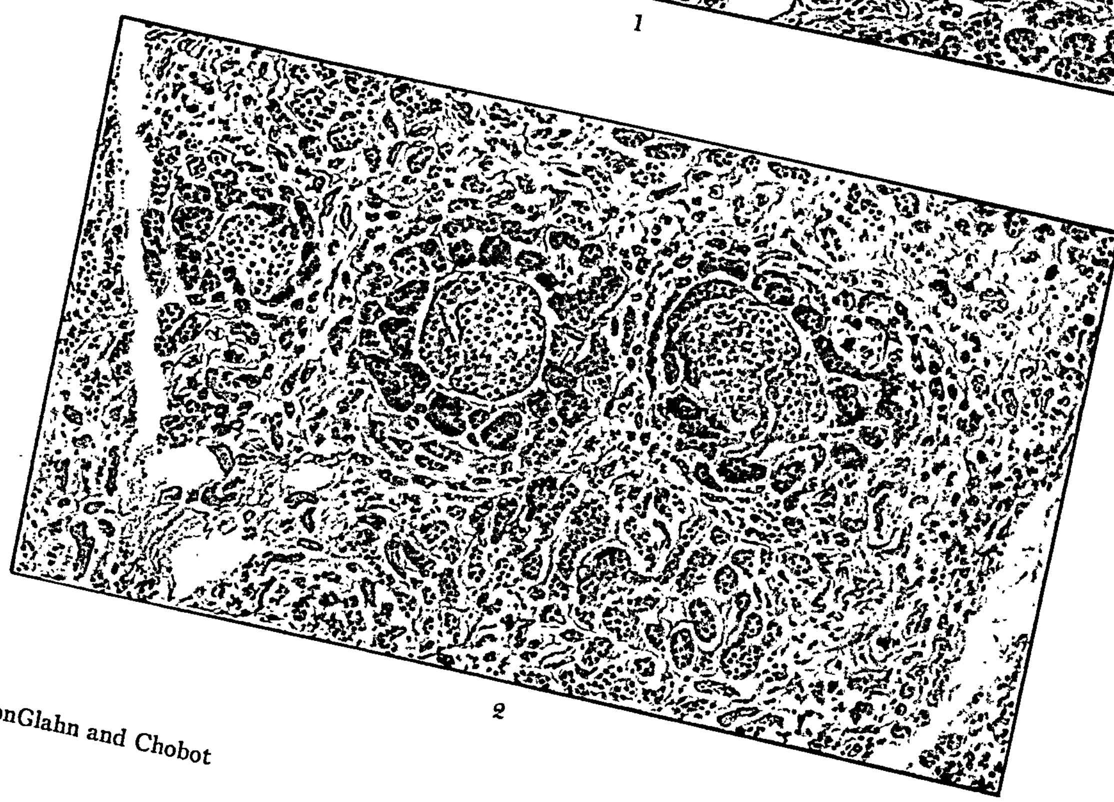
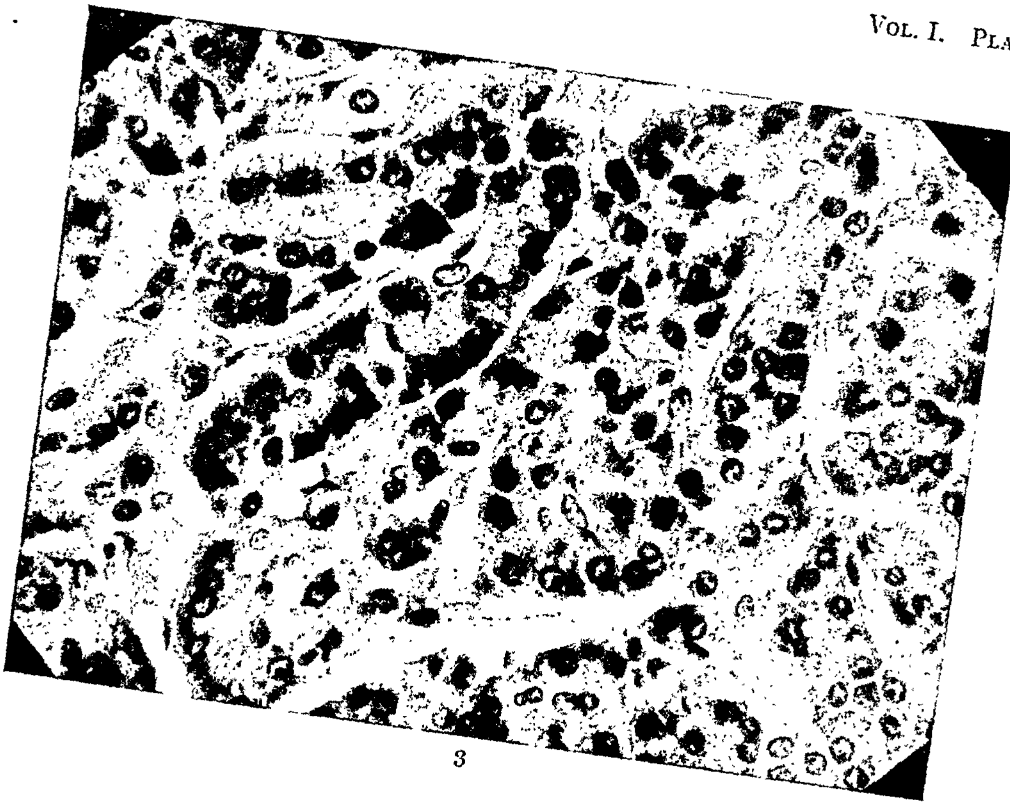


2

VonGlahn and Chobot

Pancreas in chronic passive congestion



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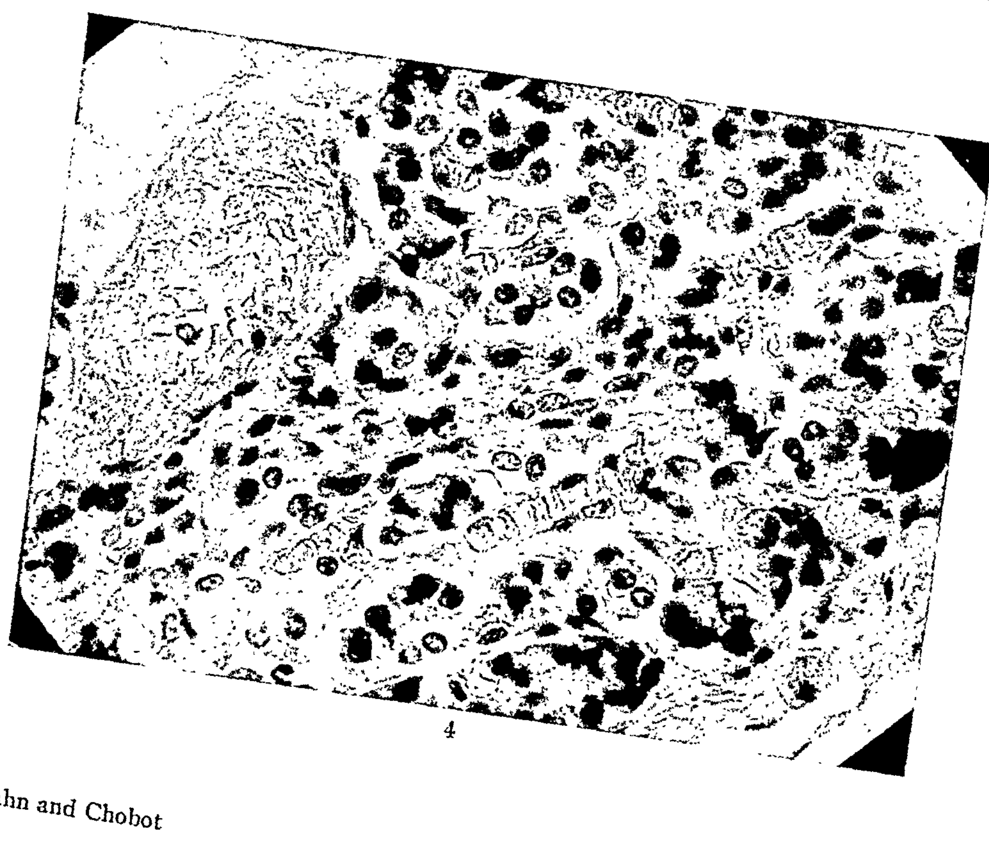

4

VonGlahn and Chobot

Pancreas in chronic passive congestion

CHOLESTEROSIS OF THE GALL BLADDER *

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The wall of the gall bladder is structurally well adapted for absorptive purposes. The mucosa of the gall bladder is formed of tall, thin, villous structures, intricate in their extensive branching, and delicate in the supporting framework of the stroma. If anatomic structure is the result of physiologic need, then certainly this mucosa is well formed for the end in view, that of intensive absorption. Indeed, physiologists have agreed that the gall bladder is essentially an organ of concentration. Rous and McMaster have proved definitely that water is absorbed from the bile in the gall bladder, thus concentrating the latter six to ten times. Moreover, physiologists have demonstrated the absorption of solid matter through the wall of the gall bladder. In 1920, Harer, Hargis and Van Meter injected potassium sulfocyanid into the cavity of the gall bladder of dogs and recovered the salt in the cystic node within five minutes after injection, thus showing the passage of particulate matter from the lumen, through the wall and thence into the lymphatic structures of the biliary system. In 1921, Boyd, after injecting potassium ferrocyanid into the lumen of the gall bladder of dogs, obtained the Prussian-blue reaction in the epithelial cells of the mucosa and even in the connective tissue cells of the stroma of the wall within thirty minutes.

I have repeated the work of these investigators and obtained similar results. Finely powdered carmine powder, charcoal and India ink were also used in separate experiments. Solid particles of these were found in the lymphatic structures within a few minutes after the injection into the cavity of the gall bladder.

The passage of solid substances, thus demonstrated, lends weight to the opinions of Grigaut, Boyd, Dewey, Starling and Sweet who believe that lipoids such as cholesterol are absorbed by the epithelial cells of the mucosa of the gall bladder. On the other hand, many

* Work done in the Division of Pathologic Anatomy, Mayo Clinic. Received for publication April 6, 1925.

workers, notably Adami, Herter and Workman, believe that cholesterol is excreted by the mucosa. A parallelism is drawn between epithelial cells of various parts of the body and those of the mucosa of the gall bladder, and the presence of cholesterol crystals in epithelial structures is cited. For instance, such crystals have been found in the bronchi and their presence attributed to "excretion" of cholesterol by the epithelial cells lining the bronchi. They have been found in leiomyomas of the uterus and attributed to similar excretion there. But it seems more reasonable to believe that the cholesterol crystals found under these circumstances are the result of cellular disintegration, as the cholesterol found in arteriosclerotic patches of the aorta undoubtedly is.

The experimental data recorded here are positive evidence of actual absorption of lipoid material by the epithelial cells of the mucosa of the gall bladder. So far as I know there is no other experimental evidence substantiating this work, but numerous investigators have said that cholesterol is probably absorbed by the wall of the gall bladder. In March, 1924, at a staff meeting at the Mayo Clinic, I reported the following experimental data:

Laparotomy was performed on twenty-three dogs anesthetized with ether. Into the cavity of the gall bladder of four of the dogs, finely powdered carmine suspended in distilled water was injected through a fine hypodermic needle in amounts of 10, 15, 20 and 25 c.c. These animals were necropsied within five minutes after the injections and in each carmine was observed in the cystic node. Other similar experiments were performed, powdered charcoal and later India ink being used in each case, with the same recovery of particulate matter in the cystic node. Therefore it seemed justifiable to conclude, as had previous investigators, that the wall of the gall bladder is capable of absorbing solid substances as well as fluids.

These findings led to experiments with other substances. The absorption of fats by the wall of the gall bladder proved an inviting problem and the experiments were repeated, using lipoid substances instead of particulate matter. Dogs were anesthetized with ether, and highly emulsified Sudan-stained fat particles injected into the cavity of the gall bladder. But in no instance was microscopically demonstrable fat discovered in the cystic node, even several hours after the injection. However, fat-stained particles in fresh untreated sections were present in the epithelial cells of the mucosa of

the gall bladder within a half hour after injection. And at varying intervals up to three hours, similar Sudan-stained globules were found in the connective-tissue cells of the stroma, and even in vascular endothelial cells. Similar observations were made by MacCarty in 1919 in a study of "strawberry" gall bladders. However, similar experiments with milk, olive oil and cholesterol esters were without success; in no instance was it possible to recover lipoid substances from the lymphatic structure of the wall of the gall bladder, even several hours after the injection.

However, the use of highly emulsified milk fat, deeply stained orange with Sudan III, produced astonishing results. Laparotomy was performed on anesthetized dogs and the fat-stained emulsion injected into the cavity of the gall bladder as in the previous experiments. Twenty-five minutes after the injection minute Sudan-stained globules were seen in the epithelial cells of the mucosa of the gall bladder, in otherwise unstained sections. And within one hour after injections these stained microscopic lipoids were seen as multiple, discrete masses throughout the epithelial cells, most numerous at the periphery of the cells, but present also at their base. In other sections, taken from the gall bladder of dogs necropsied two and three hours after injection, these tiny globules of previously stained fat were seen in the stroma and even endothelial lining of the blood vessels. Similar experiments, using cholesterol crystals dissolved in oleic acid, were not so satisfactory, but were sufficiently conclusive to verify the previous observations.

From these data, it was concluded that the wall of the gall bladder had the power to absorb lipoid substances, and that this absorption was effected, probably, by a direct passage of the lipoid through the epithelial cells of the gall bladder mucosa. Furthermore, it seemed likely that lipoid substances differed from particulate matter, in that they were not swept into the lymphatic stream, but were carried away through the blood vessels. At least, sufficient data were present to indicate that lipoid substances might pass through the wall of the gall bladder from its cavity.

The passage of lipoid through the wall of the gall bladder is probably a metabolic one, but our knowledge of the metabolism of lipoids is too indefinite to warrant any further speculation on this point. Suffice to say, physiologists are not in accord with the present theory of lacteal absorption of fats from the intestinal tract. Indeed, Mac-

Callum has proffered the suggestion that fats are probably absorbed directly by epithelial cells, and that this may be the method of their passage from the intestinal tract. However this may be, the factors concerned in such a passage are probably easily disturbed, and this disturbance would probably result in imperfect or incomplete passage of the lipoid through the wall of the gall bladder, with their resultant accumulation in the wall. Undoubtedly the lipoids would first accumulate in the epithelial cells of the mucosa, and later pile up in the connective-tissue cells of the stroma of the villi. Indeed, this is the characteristic picture of strawberry and papillomatous gall bladders on microscopic examination. The former shows the lipoid scattered diffusely over the surface of the mucosa, involving the distal or proximal portions of the epithelial cells and later the structure of the villi themselves. The papillomatous organ presents essentially the same picture, except that the areas of gross lipoid are localized in discrete polypoid masses.

However much we may be concerned with the direction of the passage of lipoid through the wall of the gall bladder, whether it passes from within the cavity into the wall, or vice versa, we cannot escape the fact that lipoids accumulate in the structures of the wall itself. Indeed, it is quite possible that the lipoid thus seen in the strawberry and papillomatous gall bladders is but an accumulation of cellular lipoid resulting from the degeneration of the cells themselves. It is difficult to believe such a contention, however, when one sees the relatively huge masses of lipoid that often distend the frail polypoid villi. It seems scarcely possible that such an amount of lipoid could come from the degeneration of the relatively few cells constituting their histologic structure.

Regardless of the method of accumulation, however, the fact is established that the gross appearance of the strawberry, fishscale and papillomatous gall bladders is due to lipoid substances piled up in the structures of the wall of the gall bladder, principally in the mucosa. MacCarty, in 1919, demonstrated that these lipoids are essentially cholesterin esters, and numerous observers have since verified this.

We are concerned, then, principally with an alteration in the cholesterol metabolism occurring in the wall of the gall bladder. The disturbance may be purely local, confined, so far as can be determined by our present methods, to the wall of the gall bladder itself.

That it is often associated with generalized disturbance in the fat metabolism of the body seems true.

In a study of 633 consecutive necropsies, in which I gathered the data myself, thereby reducing to a minimum the personal equation, 134 strawberry gall bladders, sixty-one papillomatous, and twenty-nine showing both conditions, were found. None of the persons was under fifteen years, and only three were under twenty; thus 34.7 per cent of adults showed this fat disturbance in the gall bladder. Also 21.6 per cent of the adults had gall stones, and 30 per cent of these also had fat alteration, making a total of 46.4 per cent of persons over the age of twenty that had some gross evidence of accumulated lipoid in the wall of the gall bladder. The lesion was found in 45.7 per cent of 150 consecutive men, whereas it was found in 53.9 per cent of women.

A majority of persons with fatty changes in the wall of the gall bladder also had fatty changes in other portions of the body. For instance, sixty-seven women had pathologic lipoid changes in the mucosa of the gall bladder. Fifty-eight of these (86.5 per cent) had been pregnant. It is well known that pregnancy is associated with fatty changes throughout the body, and that disease of the gall bladder frequently accompanies pregnancy. Likewise, the fat disturbance occurring in cases of obesity seems to be associated with the lipoid disturbance occurring in the wall of the gall bladder. In fifty-one necropsies, the subjects weighing over 200 pounds, 78.4 per cent had gross fatty changes in the wall of the gall bladder, and 94 per cent had abnormally large amounts of microscopic lipoid.

There is a slight increase in the frequency and amount of fatty changes in the liver in cases of lipoid disease of the gall bladder; the significance of this is not known. In 54.2 per cent of 315 cases in which there was no lipoid visible in the wall of the gall bladder, there were fatty changes in the liver, whereas in a series of 233 cases of strawberry and papillomatous gall bladders, fatty changes were present in the liver in 68.2 per cent. This is only a 14 per cent increase in the frequency of fatty changes in these cases, but the much greater extent and severity of the process was more striking.

That other organs in the body are concerned with these fatty changes is probable. It is known that certain organs contain relatively high proportions of lipoids, particularly the brain, adrenals, liver and blood vessels. Moreover, it is known that the cholesterin

content of these organs varies considerably, and probably fluctuates from day to day. Certainly it is true that the cholesterol content of the blood and bile varies from hour to hour and can be readily influenced by fat food intake. It seems likely that the various organs of the body are influenced by the lipoid content of the blood, and therefore change their lipoidal content as does the blood.

To facilitate the study of lipoid changes in the body, and especially that of cholesterol metabolism, and to obviate the rather unscientific terms of "strawberry" and "fishscale" gall bladders, a term descriptive of these changes has been sought. Inasmuch as it has been proved that the lipoid disturbance seen in the strawberry gall bladder, and the similar conditions, fishscale and papillomatous gall bladders, is essentially a cholesterol one, and inasmuch as the cholesterol content of the brain, adrenal, liver and blood vessels at least, undergo quantitative changes, it seems advisable to select a name, scientific and universally applicable, that will be descriptive of such alterations. These conditions are fulfilled by the term "cholesterosis," which is formed from the Greek "kolesteron" and "osis," meaning respectively "cholesterol" and "dysfunction." Our present knowledge does not permit further speculation on the type of changes that occur in the fatty derangements of the body. But we know that the pathologic changes here referred to are concerned with cholesterol, and the word "cholesterosis" simply indicates this dysfunction without further commitment. Regardless of what future studies may reveal as to the mechanism of this dysfunction, we know that the disease is concerned with cholesterol, and so the term will always be appropriate.

ARACHNID INFECTION IN MONKEYS *

(Pneumonyssus Foxi of Weidman)

Ferdinand C. Helwig, M.D.

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During the course of routine autopsies upon monkeys used in the Department of Experimental Medicine at the University of Kansas, six animals from a consignment of nine were found to possess peculiar pulmonary lesions which were at first mistaken for tuberculosis. Further examination, however, showed them to be caused by an arachnid parasite which was discovered by macerating material from the lesions in a strong solution of potassium hydroxide and examining without staining. From every lesion examined one or more arachnid parasites were recovered.

Of the six animals possessing these lesions, two were used in the production of an experimental lobar pneumonia and one underwent an operation in which high obstruction of the ileum was performed. This animal died about 36 hours later. The remaining three showed no other gross lesions aside from the pulmonary infection. The monkey dying of intestinal obstruction showed in addition in the terminal ileum and colon, nodules varying in size from 6 to 14 mm. in diameter, extending above the surface on both the peritoneal and mucosal surfaces from 3 to 5 mm. From each of these nodules, nematodes were obtained which varied from 10 to 16 mm. in length.

Gross pathology. The lung lesions were subpleural, cone-shaped sacs which extended above the surface of the pleura from 0.5 to 1 mm. The larger lesions were slightly umbilicated on top, the smaller ones being smooth. They showed a yellowish gray center with a bright yellow border. The edges of the lesions were somewhat indurated and after opening revealed cavities with a thick wall of chronic granulation tissue partly filled with yellowish dêbris. Under the dissecting microscope, numerous arachnid parasites could be seen lying free in the cavity or attached to the walls, often

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surrounded by cheesy yellow material. The number of parasites in the cystic cavities varied considerably, as many as twenty being found in one of them. Each lung contained from twenty to forty lesions varying in size from 2 to 7 mm. in diameter on the pleural surface and extending into the substance of the lung from 3 to 7 mm. It was difficult to say whether they were in direct communication with the bronchi or not but careful dissection showed that in most cases the small terminal bronchi led up to the tip of the cone-shaped sac which pointed toward the hilum of the lung suggesting that the cyst was a primary dilatation of the bronchus in which the parasite had been lodged and which was shut off from the proliferating parasites by the inflammatory capsule formed.

Histological pathology. The microscopic study of these sacs shows that they have a more or less characteristic structure, being surrounded by atelectatic alveoli with thickened walls, blending into the substance of the cyst wall. The latter is composed of three layers, in most cases rather distinctly outlined but showing a tendency for the outer two layers to fuse together. The outermost layer is made up of some young connective tissue cells, fibroblasts and round cells in which there are scattered polymorphonuclear leucocytes. Rather large clumps of golden yellow pigment and dirty yellow pigment are irregularly distributed especially toward the inner wall. Many young capillaries, often filled with red blood cells are in the middle layer. The latter is much more cellular than the others, there being many large and small mononuclear cells and some polymorphonuclear leucocytes. The inner wall is again more fibrous in character there being a marked preponderance of fibroblasts and young connective tissue. Along the inner border parasites may be seen growing into the walls of the cavity. They are in many cases apparently becoming re-encapsulated by the connective tissue reaction. The intestinal canals of these parasites are filled with granules of yellow pigment indicating that they are probably parasitic excreta. This tendency to re-encapsulation is noted in numerous instances and is verified by the fact that in the larger cysts sectioned, the parasites are seen lying in small separate capsules in the immediate vicinity of the larger cysts. Although no organisms were found lying in bronchi which possessed intact bronchial epithelium the supposition that these organisms probably

do grow out from the bronchi is partially substantiated by the evidence of large amounts of pigment lying in the bronchi and by the encysted parasites growing out from the side of a bronchus. Both Weidman[12] and Landois and Hoepke,[11] who have described somewhat in detail cases of a similar character to these under discussion, did not find the parasites in the bronchi but Weidman[13] mentions a lesion, "abutting directly upon a bronchus with an especially necrotic wall."

The pulmonary lesions of the monkey showing the nematode infection of the gut present some scarring and more marked umbilication of the pleural covering of the sacs.

Section through these sacs shows an inner wall of more mature fibrous tissue than was noted in the previous cases and there is a more intense accumulation of round cells in the mid-zone with again a more adult fibrous tissue appearing in the outermost zone. There is a much larger accumulation of pigment in the vicinity of these sacs and it is here that the tendency to outpocketing is more noticeable than elsewhere. Some of the parasites are surrounded completely by a new capsule with a small communication which is not in any way large enough to permit entrance into the original or mother sac.

Mode of entrance. Our histological findings would tend to substantiate Weidman's assumption that the organisms probably gain access to the lungs through the air passages, lodge in the smaller terminal bronchi, proliferate, form sacs and from there burrow through into the interstitial tissue of the lung. The marked tendency on the part of the lesions to outpocketings, the parasitic excreta found in the bronchi and the suggestive location of the living organisms mentioned in the histological discussion seem to contradict the contention of Landois and Hoepke, that the ova are swallowed and gain entrance to the lung as embolic ova from the lymphatics of the intestine where they have burrowed and proliferated. As Weidman mentions the ova are of too large a size, measuring about 0.4 mm. in length, hence it seems probable that this latter source may be eliminated as a portal of entry. Further substantiation is found in Martin's case wherein he observed the mites crawling into the bronchi from the sac, and in serial sections he demonstrated bronchi leading into the subpleural cavities.

Classification. Although the organism somewhat resembles both that described by Hoepke and also Weidman, we felt that the matter of classification demanded the attention of an expert parasitologist and as a result some of the tissue was sent to Dr. H. B. Hungerford[17] of the Department of Entomology at the University of Kansas, who reported it to be the Pneumonyssus Foxi of Weidman. Before sending this material to Dr. Hungerford we thought that it might be of a somewhat different species than already described, due to the fact that the tissue reactions varied to some extent from those mentioned; this, however, might be accounted for by taking into consideration the age of the lesions. Dr. Hungerford sent some of this material to Dr. William A. Riley at the University of Minnesota who concurred with him in the diagnosis.

The following is a brief description of the organism after Weidman. "P. foxi n. sp.; diagnosis: Adult females, yellowish white, opaque, in width a little more than half the body length. Dorsal shield with ten hairs and pitted areas, ventral with six hairs. Anal plate present with three hairs. All tarsi furnished with two dorsal claws, all except leg i with caruncle in addition; all articles hirsute and most also spinulose. Both tarsi and femora subdivided. Palpi of three segments, all short, the terminal one capped by a short bristle. Mandibles chelate in both sexes. One pair of stigmal plates between and dorsal to coxae iii and iv. Hypostome bears a median longitudinal row of 9 to 13 teeth, carries ten hairs anterolaterally and four anterior marginal papillae. Vulva short, median and fissural at level of coxae iv. Adult males measure a little less than half as wide as long. Sexual orifice circular and close behind capitulum. Larva hexapod, oval, 0.55 × 0.28 mm., bears anal plate. Length 0.875 mm., breadth 0.478 mm.

"Habitat, lungs of monkey."

Discussion

Weidman does not feel that this organism is of high pathogenicity and fails to mention any pulmonary symptoms in conjunction with the infection. Three of our six cases, which did not show any other anatomical lesions at autopsy, during the course of their pulmonary infection were subject to very frequent attacks of paroxysmal coughing and sneezing, which we attributed to the arachnid infection. In addition, the fact that the lesions were more widespread

than those previously reported and that they caused, as we believed, such prominent respiratory symptoms, coupled with the additional finding of pulmonary edema in the uncomplicated cases, led us to conclude that they were of more than passive importance in bringing about the fatal termination.

Infection in man. It is of some interest to note that the arachnid infection of the internal organs of man is not, as is commonly believed, entirely unknown. A review of the literature brings to light a number of authentic cases and several in which the matter of contamination might be brought forward as an objection. The first case is that reported by Miyake and Scriba[1] in 1893, in which twenty-five mites and six eggs were found in the urine of a thirty-seven year old Japanese, suffering from hematuria. Marpmann[2] in 1898 found a solitary mite in the urine of a patient with gravel and albumin in his urine. Huber[3] in 1899 tells of three cases, one the case of Reinhart in which an arachnid was isolated from vomitus of a woman suffering with carcinoma of the stomach, the second that of Leroyde de Mericourt which showed parasites in the pus from a draining ear and the third that of Lambl's in which organisms were isolated from the stool of a patient suffering with dysentery. Troussart[4,5] in 1900 discovered a male, female and larval arachnid in a testicular cyst which was of six years' duration. De Haan[6,7] in 1906 found them in large numbers in the urine of one of his patients. Castellani[8] in 1907 while doing an autopsy upon a Beranda native in the Uganda discovered an arachnid parasite in a small cyst embedded in the omental fat. In 1910 Tieche[9] found them in the stools and urine in a case of erythema multiformis. Again in 1910 Tsunoda,[10] while examining a stool from an anemic patient in Japan, saw large numbers of arachnid parasites in all preparations studied. Recently (1921) Dickson[15] has reported two striking cases of mites in the urine, one in a patient with a severe cystitis and the other in which arachnids were said to be derived from the right kidney. The authenticity of some of these cases will perhaps be questioned but many of them seem to defy contradiction, especially those published more recently.

The possible advantage in reporting this apparent epidemic among a consignment of monkeys is to impress the possibility of internal infection by arachnid parasites in the human. The nature

of the lesion is essentially a local one suggesting the inability of the body to destroy the parasite and the necessity of surrounding it with fibrous granulation tissue to check its further spread. Undoubtedly the persistent cough of the animals was due to the irritation of these encysted organisms.

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DESCRIPTION OF PLATES LXI–LXII

Fig. 1. Photograph of pleural surface of lung, light area at apex shows external appearance of lesion. Natural size.

Fig. 2. Photograph of cross section through lung showing two communicating subpleural sacs. Natural size.

Fig. 3. Photomicrograph of section showing organism burrowing into wall. H and E stain. x 80.

Fig. 4. Photomicrograph of immature mites. x 60.

Fig. 5. Photomicrograph of section through a sac showing organisms lying within. x 8.

Fig. 6. Photomicrograph of six-legged Larva. x 60.

Fig. 7. Photomicrograph of adult female Arachnid. x 100.

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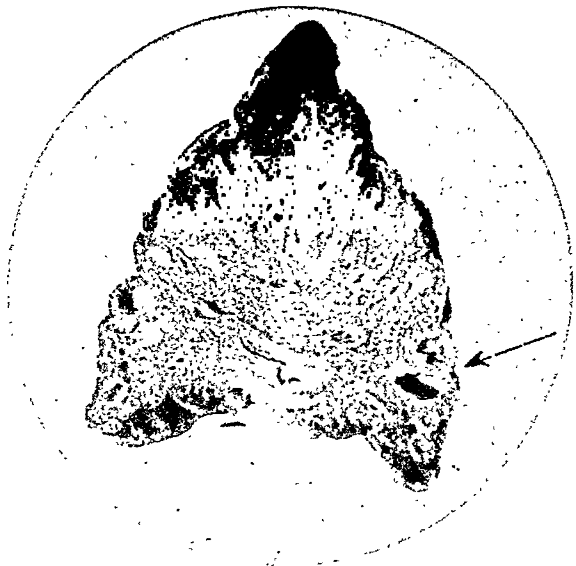

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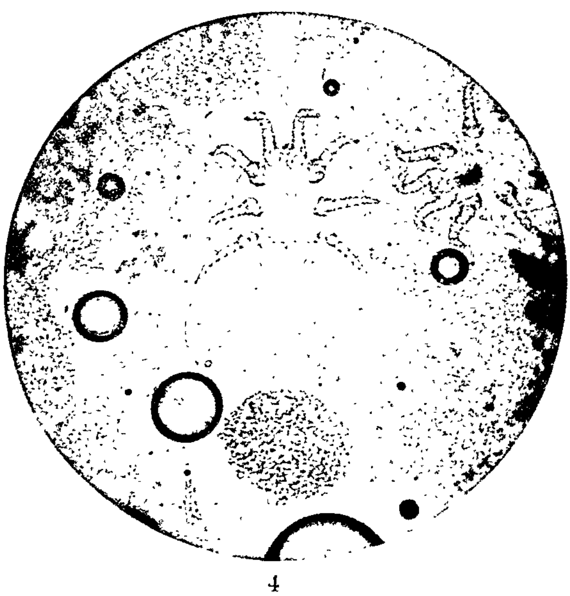

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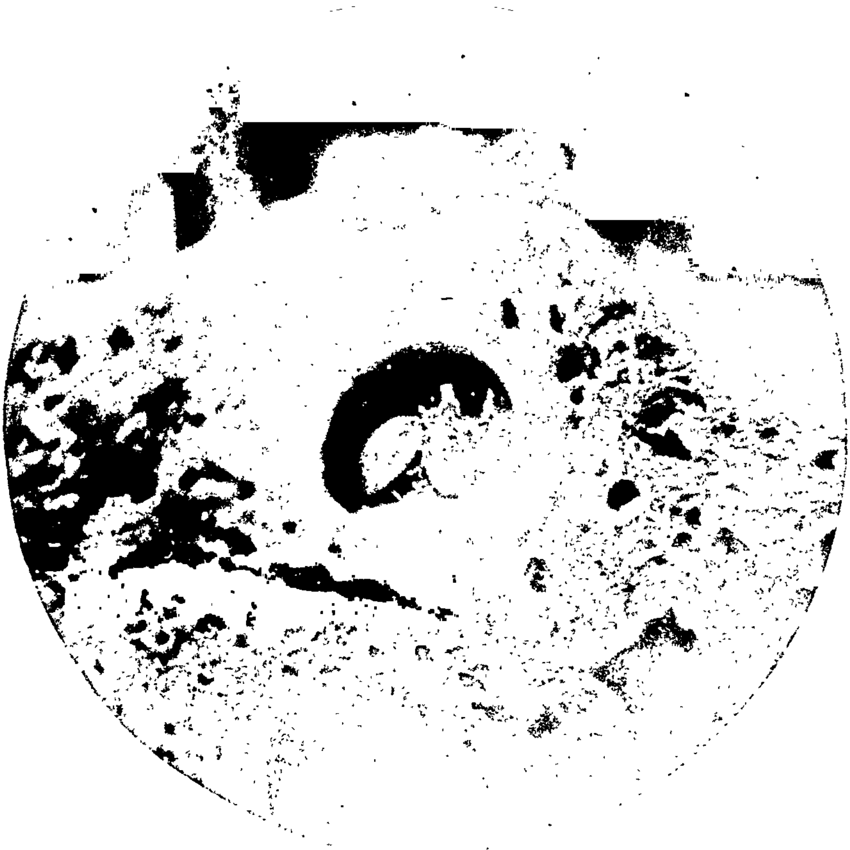
Helwig

Arachnid infection in monkeys



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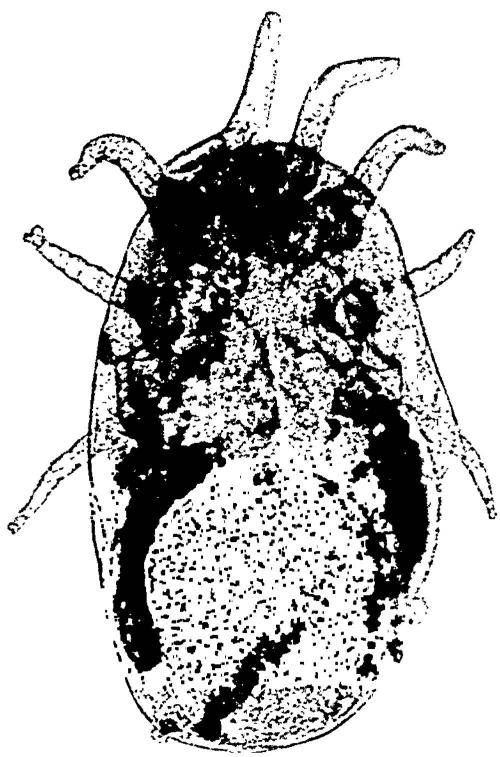

7

Helwig

Arachnid infection in monkeys

MUCOCELE OF THE APPENDIX WITH GLOBOID BODY FORMATION *

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Cystic dilation of the vermiform appendix is an infrequent but interesting pathological entity, of which 168 cases are recorded in the literature. A more rare form of the same condition is the so-called "fish-egg" or "globoid body" type, of which there are only six cases reported. Because of its rare occurrence we wish to record another interesting example illustrating this unusual disease.

Latham [1] reported a case which consisted of a dilated appendix 9 cm. in length. There was no opening between the appendix and cecum, the normal orifice having been closed by an obliterative enteritis. The structure was found to be filled with small round transparent bodies the size of dried peas. Sections revealed a fibrous-like structure with an irregular distribution and laminated in parts, being more laminated at the circumference where it had the appearance of a capsule. These sections stained with acid dyes and gave no reaction for mucin; they did not stain by the Weigert method for fibrin. The wall of the appendix was irregularly honey-combed by the pressure of the bodies; it was stiff and thickened, the mucous membrane had almost disappeared and no lymphoid tissue remained. The condition had given no symptoms during the life of the patient who was a man aged 46. He died of lobar pneumonia.

Another example of this same lesion is mentioned by Cobbett and Keilin.[2] Dr. John Pooley of Nottingham sent the specimen to them and appended a brief statement of the case. The patient was a soldier who had died of carcinoma of the intestine with metastases in the pancreas and suprarenal bodies. There were no signs or symptoms of a diseased appendix and it was opened at necropsy only because it was distended. "It was so kinked that its cavity was quite shut off from the rest of the bowel." Upon opening the appendix the content was found to be many small grayish-white bodies

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that were at first considered to be parasites. They resembled frogs' eggs very closely, except that the nuclei were whitish rather than pigmented, and they were not adherent one to another. They appeared spherical when floated in a weak solution of formalin, but some of them were drawn out to a blunt stalk-like point on one side. The size varied, the majority measuring from 2.5 mm. to 3 mm.; some were even 4 mm. in diameter. Their outline was sharply defined; in the middle of each could be seen an opaque white nucleus, measuring about half the total diameter, and outside this, a peripheral zone which was gray and translucent. Sections of these bodies showed that they were devoid of structure, beyond the fact that the nucleus was denser than the remainder and torn away from the peripheral zone.

A second case was noted by Keilin [3] in the First Eastern Hospital, Cambridge, during the war. This was found at autopsy in a soldier who had died of an infected wound. The appendix had given no signs or symptoms during life. The writers do not give a detailed description of this case.

Shattock [4] reported a case in a male, age 23 years, who had symptoms of appendicitis and was operated upon. The appendix was removed. It was somewhat enlarged and was tightly packed with semi-transparent spherical bodies "like the roe of a fish." There were numerous patches of inflammation on the peritoneum. When crushed and stained with iodin the bodies exhibited groups of oval nuclei distributed in a homogeneous matrix. Serial sections including the wall of the appendix and the adjacent contents led this writer to state, "There is a catarrhal inflammation, accompanied by a hyper-secretion of mucus and desquamation of the proliferating epithelial cells, the mucin affecting the form of spherules as though extruded from and accompanied by a destruction of the crypts."

Morrison [7] recorded another very interesting example of this type of mucocele, discovered while operating upon a patient for chronic cholecystitis. The appendix was found to be markedly dilated, extending into the true pelvis.

The gross description of the appendix given by him is as follows: "The appendix appeared whiter in color than the normal; it was of firm consistence, as if distended with a solid substance. There was a uniform enlargement from base to tip, and its diameter was as large as the width of the small intestine. A few adhesions were pres-

ent about the appendix. At its base, where it joined the cecum, there was a definite constriction of the appendicular walls. The mesentery appeared normal."

The pathological report of the specimen made by Dr. F. B. Mallory of the Boston City Hospital is as follows: "The length and width of the gross specimen measured 5.5 centimeters long by 2 centimeters in diameter at its base, and 1.5 centimeters in diameter at its tip. A transverse incision was made at the middle of the appendix, and another longitudinal incision was carried through its walls opposite the mesentery. The lumen was distended with a quivering, jelly-like mass, embedded in which were innumerable small rounded nodules, varying from a translucent to a white color and up to one millimeter in diameter, the whole resembling fish roe; the substance was not attached to the wall of the appendix, merely distending its lumen. When dropped into alcohol, formaldehyde-alcohol, Zenker's and Klotz's fluids, the little bodies immediately shot away from the mass. The appendiceal wall was about one to two millimeters thick, pale and white; it had two firmer nodular thickenings, each about four millimeters in diameter, at the mesenteric attachment.

"The microscopic examination of the wall of the appendix in its thinnest portion showed almost complete atrophy of the mucosa, with disappearance of the epithelial elements. No inflammatory reaction was evident. The muscle coat was intact, but stretched and thinned. The small balls of secretion were composed of masses of necrotic cells embedded in mucus. A section through the proximal end of the appendix showed the mucosa present, but the glands were obliterated, probably owing to the stretching which had taken place. The lining epithelium was thickened to form a stratified layer from three to eight cells in thickness. The cells were of cylindrical type, and a few of them were multinucleated (half a dozen to a dozen nuclei in each). The process resembled that sometimes seen in the gall bladder when stones are present.

"The specimen represents an advanced stage of cystic dilatation of the appendix, in which not only mucus is found, but also little balls of mucoid material, which may later become calcified, as well as the walls of the appendix itself. For descriptive purposes it may be called a fish-egg mucocele."

We are also indebted to Dr. F. B. Mallory for directing our atten-

tion to the reference to another case of this variety of mucocele that was reported by Cagnetto,[8] a very similar type of lesion to that found by Morrison in his case. This was also an accidental finding and the patient had no symptoms referable to the vermiform appendix.

To the foregoing cases we wish to add one for which we are indebted to Dr. R. W. Noble of Temple, Texas.

Case Report

Mrs. A. G. Age 32 years.

Summary. Recurrent right lower abdominal distress; tenderness upon deep pressure over McBurney's point; left lower abdominal pain; surgical intervention with removal of a simple cystic left ovary, chronically inflamed right fallopian tube and dilated appendix filled with "globoid bodies" composed of pseudomucin; lumen of appendix closed at cecal junction.

The patient was admitted to the King's Daughters Hospital, Temple, Texas, on March 7, 1923. She complained of an attack of pain in the right iliac region. This pain was a recurrent one felt at intervals for several years. There was pain and tenderness in the left side of the lower abdomen.

The physical examination revealed some pain upon deep pressure over McBurney's point. A bimanual examination of the pelvic structures brought out the presence of an enlarged and painful cystic ovary on the left, as well as a laceration of the cervix and a laceration of the perineum.

The white blood count was 5,400. There were 64% polymorphonuclear leucocytes. There was not an eosinophilia.

A cystic left ovary, the right fallopian tube that was the seat of chronic inflammation and a very markedly dilated vermiform appendix were removed at operation by Dr. R. W. Noble.

The patient made an uneventful recovery, and when seen by Dr. Noble in July, 1923, she was in perfect health.

Pathological Report

The appendix is larger than normal, being 10 cm. long and 4 cm. in diameter at the widest point. It is tapered from the cecal end toward the tip. (Fig. 1.) The peritoneal surface is smooth and has retained the normal sheen. The structure is entirely filled with small globoid bodies that are semi-transparent. Many of these bodies have a denser, whiter central spot; further, all of them have a tapered tip giving the appearance of small pears. The size of these bodies varies widely, the smallest being no larger than the head of a pin, while the largest is 1 cm. in diameter and 0.9 cm. in length. No connection exists between the various structures as is evidenced by

their freedom when placed in water. Chemically we find that they are composed of pseudomucin.

The appendix and the globoid bodies were fixed in formalin. Sections stained with hematoxylin and eosin bring out the fact that the wall of the appendix is greatly thickened, the chief constituent being fibrous tissue, which in some places has become hyalinized. Very little remains of the mucosa and the submucous coat is very much thickened and infiltrated with small mononuclear leucocytes. The serosa is not remarkable. (Fig. 2.)

The globoid bodies have been studied by serial sections and their structure found to be of granular and fibrillar form. (Fig. 3.) The granular material takes the basic stain suggesting a chromatin nature while the fibrillar portion has an affinity for the acid dye. An attempt to section one of the bodies and the appendiceal wall at the same time proved to be a failure, the friability of the tissues being too great.

The central opaque area seen in the gross examination of these bodies is not of different nature from the peripheral zone, but is more compact.

Discussion

The etiology of mucocele of the appendix is rather obscure. Elbe [5] has given some underlying conditions that are essential for its development.

First, a slowly stenosing process at one or more points of the lumen. A rapid stenosis produces gangrene.

Second, a sterile lumen must be present distal to or between the points of stenosis. Bacterial invasion, if of sufficient virulence, would cause a secondary empyema of the appendix.

Third, there must be an actively secreting mucosa, or at least a more rapid secretion than absorption, and also some change in the mucosa whereby the normal mucus is transformed into pseudomucin.

In the globoid body form these features are essential but an additional one is also present, the mucoid material being excreted from the crypts, as suggested by Shattock.[6] An inflammatory reaction is of considerable importance as a stimulus to hypersecretion of mucin, and all of the cases collected that were studied microscopically presented this feature except the one reported by Morrison.

Conclusions

1. Mucocele of the appendix is very infrequent.

2. The globoid body type of mucocele of the appendix makes up only 0.35 per cent of the total number of reported cases.

3. This unusual form most probably represents a combination of secretion of mucus into the crypts and some disturbance of reabsorption of the secreted material, plus a chemical change whereby normal mucus is transformed into pseudomucin.

4. Less than half of the reported cases had symptoms referable to the appendix during life, the condition being frequently an accidental finding at necropsy or during an exploratory abdominal operation.

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DESCRIPTION OF PLATES LXIII–LXIV

Fig. 1. Globoid bodies in a mucocele of vermiform appendix. (Case of Milliken and Poindexter.)

Fig. 2. Section through the wall of the appendix showing a crypt filled with mucoid material. (High power photomicrograph.)

Fig. 3. Section of a globoid body demonstrating the granular structure of this. (H. & E. stain, high power photomicrograph.)

Fig. 4. Fish-egg or globoid body mucocele in the vermiform appendix. (Case of Morrison.)

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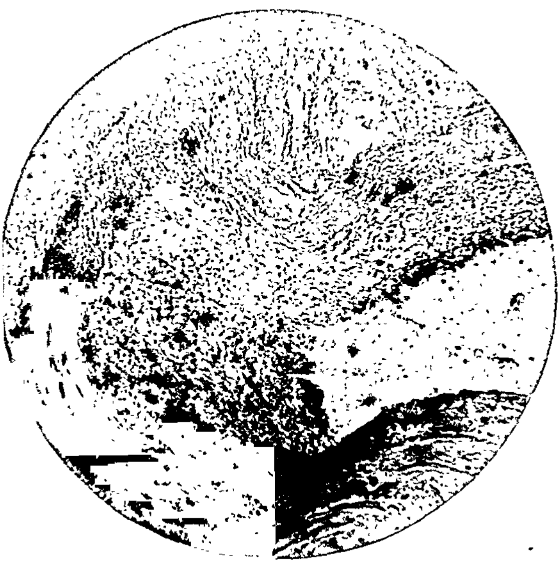

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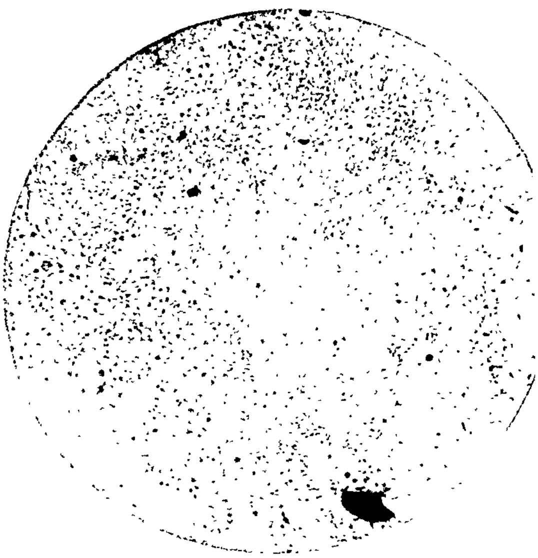

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Milliken and Poindexter

Mucocele of the appendix

4

Milliken and Poindexter

Mucocele of the appendix

THE EFFECT OF CERTAIN METABOLIC CHANGES UPON THE AORTA OF RABBITS AND GUINEA PIGS *

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Introduction. There are recorded in the literature a great number of attempts, with varying success, to produce experimental arteriosclerosis in animals. Numerous investigations have been conducted on an empirical basis. Many of the experiments which have been successful in the production of arteriosclerosis have at least one factor in common: that is, the arterial lesions have been observed in animals following the ingestion or injection of substances which alter metabolism. There would therefore appear to be some possible relationship between metabolic alterations and the experimental production of arteriosclerosis.

Pick and Pineles [1] reported that the production of arteriosclerosis in animals by adrenalin is favored by the feeding of thyroid substance. Murata and Kataoka [2] observed that the adrenalin type of arteriosclerosis occurred frequently in animals fed on thyroid substance. It has been demonstrated by Williams, Riche and Lusk [3] that protein feeding definitely increases the metabolic rate and Newburgh and Clarkson [4] have reported that rabbits fed on a high protein diet show a tendency for the development of arteriosclerotic lesions. Anitschkow [5] has shown that the administration of cholesterol, which is a metabolic stimulant, may produce arteriosclerosis in animals without the occurrence of cholesterolemia.

The following study was undertaken to observe on the aortae of animals the effect of repeated administration of substances which alter metabolism, over a prolonged period of time.

Experimental. Three substances were employed, each of which has somewhat different effects. Thyroxin was used for its stimulating effect on metabolism. Sodium acid phosphate was used to produce acid intoxication, at first in doses which Spiro [6] found sufficient to produce acid intoxication and subsequently in larger doses.

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Quinine hydrochloride was used in doses equivalent to 20 grains for man, in other words large doses which alter metabolism probably with depression.

Thyroxin tablets (Squibb) were given by mouth in doses of 0.2 mg. per kilo of body weight and the crystals intravenously in doses of 0.1 mg. per kilo of body weight. Sodium acid phosphate was injected intravenously in doses of 1 c.c. of a 3 per cent solution per kilo body weight, although later this was increased to 5 c.c. of a 3 per cent solution. Quinine hydrochloride was injected intravenously in doses of 20 mg. per kilo of body weight dissolved in a 3 per cent solution of urethane as advised by Sollmann.[7]

Observations were made on rabbits and guinea pigs. The animals were weighed before and at the end of the experiment. All animals that died during the course of the experiment were excluded. Since arteriosclerotic lesions occur frequently in rabbits, 34.77 per cent according to A. B. Miles,[8] 50 rabbits and 30 guinea pigs were used as controls. The aortae of all animals were fixed in 10 per cent formalin, embedded in paraffin and stained with hematoxylin and eosin. Blocks were taken from the ascending and descending portions of the thoracic and from the abdominal aorta.

Results

Thyroxin. Ten rabbits and 8 guinea pigs were each fed 0.2 mg. per kilo of body weight by mouth daily for 60 days. Sixteen rabbits were each injected intravenously with 0.1 mg. per kilo of body weight daily for 60 days. Half of the animals of each group were killed following the last dose while the remaining half were killed two months later. The aortae of all animals were examined and neither gross nor microscopic evidence of arteriosclerosis was found. All animals showed a loss of weight. Those which were injected showed in most instances a loss of about 30 per cent although one small animal lost only 8 per cent. Those fed with thyroxin lost from 11 to 16 per cent with one exception which lost only 2 per cent. The loss of weight of the guinea pigs ranged between 16 and 48 per cent.

Sodium Acid Phosphate. Twenty rabbits were injected intravenously daily over a period of 90 days with the following doses:

Oct. 1 to Nov. 9	1 c.c. of 3 per cent aqueous solution
Nov. 9 to Dec. 9	3 c.c. of 3 per cent aqueous solution
Dec. 9 to Dec. 19	4 c.c. of 3 per cent aqueous solution
Dec. 19 to Dec. 29	5 c.c. of 3 per cent aqueous solution

Ten of the rabbits were killed following the last injection and the remaining 10 were killed 2 months later. There were no gross or microscopical arteriosclerotic changes. The loss of weight was in the neighborhood of 10 per cent, the smallest loss being 6 per cent. Guinea pigs were not used in this series because of the difficulty of intravenous administration over a long period.

Quinine Hydrochloride. Twenty-one rabbits were injected intravenously and 8 guinea pigs subcutaneously with 20 mg. per kilo of body weight each day for 30 days. At that time the necrosis at the site of injection was so severe as to interrupt the experiment. Seven of the rabbits and 4 of the guinea pigs were killed following the last injection. Fourteen of the rabbits and 4 of the guinea pigs were killed two months later. In the abdominal aorta of three animals were found small, elevated, yellow, smooth, plaques. Upon microscopical examination hyperplasia of the intima was seen with fatty changes of the hyperplastic intima and sub-intimal layers of the media. The rabbits each lost about 20 per cent in weight and the guinea pigs about 20 per cent.

Controls. Fifty rabbits and 30 guinea pigs were examined. These were apparently normal, non-experimental animals. The rabbits ranged in weight from 1200 grams to 3900 grams and the guinea pigs from 250 grams to 680 grams. In none of the guinea pigs was there any evidence of arteriosclerosis. In the descending thoracic aorta of one and the abdominal aorta of two of the rabbits there were found arteriosclerotic lesions similar to those seen in the animals that received quinine. Microscopically, there was in one case calcification of the media of the aorta. In all three aortae there was intimal hyperplasia with fatty changes of the hyperplastic intima and sub-intimal layers of the media.

Summary

There were no arteriosclerotic changes in the aortae of the animals receiving thyroxin or sodium acid phosphate. In 3 of the rabbits injected with quinine and in 3 of the control rabbits, early arteriosclerosis of the aorta was observed. The laboratory conditions are such that none of the control animals lost weight during the period of the experiment. All the experimental animals lost weight but this can be due to loss of appetite, frequent handling, etc., quite as well as to altered metabolism.

It is safe to assume that the arteriosclerosis in three experimental animals was not due to the treatment. Therefore, in the stock employed in this laboratory about 6 per cent exhibit non-experimental arteriosclerosis.

Since thyroxin failed to produce arteriosclerosis, it seems probable that cholesterol arteriosclerosis is not due *per se* to increased metabolism.

Conclusion

Under the conditions of the experiments here recorded, the metabolic disturbances incident to the prolonged administration of thyroxin, sodium acid phosphate and quinine hydrochloride do not produce arteriosclerosis of the aorta of rabbits or guinea pigs.

Grateful acknowledgment is made of the aid rendered by Professor T. Sollmann and Dr. Alan R. Moritz.

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STUDIES ON PNEUMONIA FOLLOWING NASO-PHARYNGEAL INJECTIONS OF OIL *

G. F. Laughlen, M.D.

In the routine study of autopsy material at the Toronto, Ontario, Hospital for Sick Children, an unusual microscopic picture was observed in sections of the lung from a pneumonia case. Two days later a similar observation was made in a second case.

Brief abstracts from the histories of these cases are as follows:

Case I. This child, aged 2½ years, had an illness lasting about seven weeks. She first developed diphtheria, from which she recovered after four weeks illness, and about one week or ten days later she developed scarlet fever which was complicated by Vincent's angina. She eventually developed septicaemia and died.

She was only four hours in the Hospital for Sick Children, during which time she received sod-diarsenal intravenously, 15 minims of camphor in oil hypodermically and a transfusion of blood. Menthol in alboline † had been used as treatment of her nose and throat infection previous to her admission to the hospital. Argyrol drops had also been used in the same way but a record of the amount given could not be obtained.

At autopsy grayish red nodules of broncho-pneumonia were found in all lobes of the lung excepting the left upper lobe. There was nothing unusual in the distribution or gross appearance of the broncho-pneumonic process.

In sections of the consolidated portions of the lung, the alveoli were seen to contain three types of exudate: one in which polymorphonuclear leucocytes predominated, another consisting almost exclusively of large vacuolated mononuclear cells, and a third type in which both of these cells were intermixed.

Attention is particularly called to the mononuclear cells which made up this peculiar type of exudate and which contained unstained droplets of various sizes. Some cells contained so many droplets that they were distorted in shape and considerably swollen. A few contained no droplets but the majority contained amounts intermediate between these two extremes.

By the use of Sudan III these globules were demonstrated to be oil. These "oil containing" cells did not appear to be undergoing degeneration but were healthy, as shown by the good nuclear stain-

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† Menthol — grs. I. — Alboline oz. I.

ing even of those cells which were loaded with droplets. In those which contained much oil the nuclei were often pushed to one side, flattened, or somewhat masked. In others which contained very little the nuclei were round or oval and centrally located.

The appearance and phagocytic nature of these cells characterize them as endothelial leucocytes.

Case II. This infant had an illness lasting seven weeks which commenced with a cough and naso-pharyngitis, and which was followed in one week's time by double suppurative otitis media. In the course of her illness conjunctivitis and convulsions also developed. There was a profuse nasal discharge.

Local treatment consisted of instillations of argyrol drops in the eyes and three drops of menthol and alboline in each nostril every four hours. The menthol and alboline treatment was used for thirty-six days and until three days preceding her death.

On post-mortem examination there was well marked broncho-pneumonia of the right upper lobe and both lower lobes of the lung. As in the preceding case no unusual features were seen on gross examination of the lung, but on microscopic examination, in addition to an exudate of the usual septic type, extensive areas were found in which the pneumonic exudate consisted almost exclusively of large phagocytic endothelial cells (Fig. 1). These were loaded with droplets which were demonstrated to be oil by the use of Scharlach R. stain.

Case III. This child, three years of age, was a diabetic and in the course of her treatment she received two drachms of paraffin oil as a laxative. She was in a somewhat stuporous condition when this was administered and two days later she died in coma.

Post-mortem examination of the lungs showed broncho-pneumonia of both the lower lobes.

In the sections of the lungs oil containing endothelial cells were observed in great numbers in many of the alveoli. An ordinary septic pneumonia was also present.

On reviewing the autopsies of the previous year, about three hundred in all, another case was found in which oil had been administered through the nose and which showed the typical oil phagocytes in the lungs.

A condition similar to that in the four preceding cases was found in an adult at the Toronto General Hospital.

A man aged thirty-seven years had an illness of two years' duration which consisted of multiple paralyses of the face, arms, legs, soft palate, vocal cords and accessory muscles of the larynx. He also had dysphagia, but could swallow if food were placed well back in his mouth.

He received 1.5 oz. of liquid paraffin three times a day over a period of four and one-half months. It was administered by mouth as a laxative.

Twelve days after this treatment was discontinued he had an elevation of temperature and two weeks later showed cyanosis and physical signs of broncho-pneumonia. Eleven days later he died.

At autopsy there were areas of consolidation in all the lobes of both lungs, and the cut surfaces exuded an oily liquid.

Microscopically in addition to a septic pneumonia great numbers of the alveoli were filled with endothelial cells containing oil and in places large droplets of free oil were also observed. This stained fairly well with Scharlach R. stain.

It has been customary in many children's hospitals and among pediatricians in private practice to treat nose and throat infections by the use of drops of menthol and alboline or by the use of argyrol drops. In this paper only the five cases which gave histories of "oil treatment" are referred to in the discussion which follows.

From a study of these cases it was apparent that the endothelial cell reactions in the lungs were due to oil but it still remained to explain the source of the oil and the route by which it reached the lung alveoli and also to prove that oil would produce such reaction in the lungs of animals.

The first experiments with animals were undertaken to prove if possible that the administration of oil led to reactions in the lungs of animals similar to those seen in the clinical cases.

Menthol and alboline was administered in several ways in order to study the route by which it found its way into the lungs.

It was also necessary for comparative purposes to repeat the same experiments using a 15 per cent watery solution of argyrol instead of the menthol and alboline.

For Experiments 1, 2, and 3, three healthy rabbits were selected.

Experiment 1. One-half c.c. of menthol and alboline was injected by means of a syringe and small catheter which was passed into the trachea a distance of about 5 cm. through a tracheotomy wound in the neck. Two days later 0.5 c.c. of menthol and alboline was again injected in the same way and twelve hours after this injection the animal died.

A post-mortem examination showed the cause of death to be broncho-pneumonia. Streptococcus hemolyticus and staphylococcus aureus were recovered from the lungs. The lung alveoli and bronchioles contained an exudate of red blood corpuscles, polymorphonuclear leucocytes and mononuclear cells. In some of the alveoli, vacuolated endothelial leucocytes were present. These were shown to contain oil by the use of Scharlach R. stain. Though few in number they were of the same type as those observed in the clinical cases.

Experiment 2. One-half c.c. of menthol and alboline was administered intratracheally by means of a sterile syringe and needle every second day for one month. Altogether fifteen injections were given. The animal was then killed and examined.

The lower lobes of the lung appeared deeply congested. They were air-containing and crepitant throughout and floated readily in water. On microscopic examination, many of the alveoli, especially throughout the lower lobes, were seen to contain large phagocytic endothelial cells. The droplets within these cells gave the reaction for fat. There were no polymorphonuclear leucocytes present in the exudate. The cells within the alveoli were of the same type and character as those seen in the clinical cases (Fig. 2). The lesion appeared to be produced purely by the presence of oil and was not obscured by reaction to secondary infection.

The rabbit of Experiment 3 received 1 c.c. of menthol and alboline every second day for one month and 1 c.c. daily thereafter until it died. This was injected into the pharynx by the use of a syringe and small catheter. The catheter was inserted into the mouth a distance of about two to three inches to insure the oil being retained. No attempt was made to insert the catheter into the larynx and the opening of the catheter was at the side which rendered it unlikely that any oil would be suddenly thrown into the larynx; moreover the animal was not manipulated in any way and the oil was given with the animal on its back but it was allowed to turn over after each injection.

This rabbit died (on the day I had planned to kill it) two months and six days after the experiment was commenced. The post-mortem examination showed that death was due to broncho-pneumonia. Both lower lobes of the lung were consolidated and mottled in appearance. The greater part of these lobes was dark red in color, but here and there somewhat lighter grayish-red areas were seen. At the margins of the lower lobes and in the upper lobes, bright red areas could be seen which contrasted sharply with the darker red color of other areas. This bright color did not fade on fixing in formalin but became much more distinct, as the color of the surrounding lung tissue faded. This color was due to Scharlach R. which had been added to the last eight or ten doses of the menthol and alboline which were administered to the rabbit. The quantity of Scharlach R. was just sufficient to give a bright red color to

the oil. This served to identify on gross examination the presence of the stained oil, widely distributed throughout the lung.

These features can be made out in the gross specimen. The red color is still bright although the lungs have been kept in formalin since April 21, 1924.

On microscopic examination, a widespread broncho-pneumonia was seen throughout all lobes of the lung. The reaction was of a mixed type but the bulk of the exudate consisted of endothelial cells containing oil droplets (Fig. 3). In many places no other cells were present but areas could be found in which the exudate consisted largely of polymorphonuclear leucocytes.

Two other experiments were carried out before the termination of Experiment 3. It was the purpose in these experiments to set up a throat infection which would reproduce more closely the conditions present in the clinical cases before oil was administered.

Two healthy adult rabbits were selected and their throats swabbed with 50 per cent carbolic acid. The following day both rabbits showed a severe local reaction and were breathing with difficulty.

One rabbit was then given 0.5 c.c. of menthol in alboline dropped into the pharynx and the same amount again on the following day. This rabbit died on the third day and on examination showed a well-marked broncho-pneumonia of the septic type and numbers of oil-containing endothelial cells within some of the alveoli.

The other rabbit was kept as a control and received no treatment. This rabbit recovered.

No definite conclusions have been drawn from these two experiments because the rabbit that died had a slightly more severe reaction from the phenol than had the control rabbit. The experiments are included to show that the oil had found its way into the lung alveoli although only two 0.5 c.c. doses were given.

Experiments were undertaken using argyrol 15 per cent instead of menthol and alboline. This was considered necessary because argyrol as well as menthol and alboline had been administered in one of the clinical cases which has been described, and because argyrol has been extensively used in the treatment of nose and throat infections.

Two healthy adult rabbits were used in these experiments:

One rabbit received daily intratracheal injections of 0.5 c.c. of argyrol which was introduced through a sterile syringe and needle.

This rabbit died from broncho-pneumonia on the third day but no noticeable endothelial cell reaction was found in the lungs.

The other rabbit received 0.5 c.c. of argyrol daily, introduced into the pharynx by a syringe and catheter in the same way as the menthol and alboline of Experiment 3. This rabbit died in two months and twenty-five days. It was much emaciated and the kidneys showed a very advanced nephrosis. There were also degenerative changes in the liver, but no endothelial cells were found within the lung alveoli.

Discussion

The phagocytic cells which have been especially noted in this paper have been referred to as endothelial cells. They are apparently the only ones concerned in the disposal of oil droplets. From a study of the literature it seems clear that they are identical with cells phagocytic for coal dust and other solid particles: the so-called "dust cells." They are also the same as the endothelial cells of pneumonic exudates, the epithelioid cells of tubercles and the heart-failure cells of passive congestion of the lung.

It is also clear that besides possessing marked phagocytic power, they all possess considerable power of ameboid movement. Their origin has long been in dispute. In the present state of our knowledge there is no reason to believe that they arise from the lining cells of the alveolar walls.

Permar [1] has shown that they arise largely from blood vessels of the alveolar walls and perhaps from vessels of more distant parts such as the liver and spleen. He found that under the stimulus of foreign bodies in the alveoli the endothelial cells of vessels proliferated, developed power of ameboid movement, entered the alveoli and became actively phagocytic.

The reaction in the lungs of animals following oil administration has been shown to be identical with that seen in children.

In three of the children the only way the oil had been given was by nasal drops which were allowed to run back into the nasopharynx while the children were in bed and on their backs.

The quantity of oil in two of these cases was considerable: 6 drops every four hours or about 2 c.c. a day for a considerable period of time. In another case only a single dose of 2 drachms was given by mouth.

In the five clinical cases and two of the animal experiments, oil found its way into the terminal air passages though it was only injected into the nose, mouth or pharynx.

In the experiments where oil was administered by mouth as well as in the clinical cases the reactions to oil were complicated by septic pneumonia, but in Experiment 2 in which oil was injected by needle into the trachea a purely endothelial cells reaction with no complicating sepsis was set up.

In one rabbit a granulomatous tumor developed at the side of the mouth where the surface was saturated with oil from the injections.

These observations suggest that oil, by lowering the resistance of the tissue or by carrying infection from the nose and throat, may be a factor in the production of pneumonia.

The oil used for mouth injection and intratracheal injection was the same. It was not sterilized so that accidental infection from outside may have occurred; but in that case the rabbit of Experiment 2, that received oil by intratracheal injection and in which more oil must have entered the lungs, should have developed a septic pneumonia more readily than the others. This points to the infection being autogenous in spite of the contention that the organisms ordinarily present in the throats of man and animals are incapable of producing infection in the host.

In Experiment 3, in which unstained oil was administered for nearly two months and oil stained with Scharlach R. was given throughout the last week or ten days, there was considerable unstained oil within the lungs, showing that oil had been reaching the lungs in the earlier part of the experiment. This oil could have carried infection equally as well as the oil given in the last ten days. Apparently the animal did not develop septic pneumonia until a large number of its lung alveoli were filled with endothelial cells and oil.

The reactions in these cases were produced by mineral oils but similar reactions have been observed in the lungs of animals following intratracheal injections of olive oil.[2, 3]

The movements and final disposal of the oil phagocytes are subjects for further study.

This paper has called attention to a type of pneumonia which has not been previously reported as occurring in children.

Conclusions

These observations and experiments have established three facts:

1. That oil finds its way into the alveoli of the lung, not only when directly introduced into the trachea but also at times when administered in sufficient quantity in the nose and throat.

2. Oil when present in the lung is actively phagocyted by endothelial cells which are present in sufficient numbers to dispose of all the oil present and produce consolidation of the lung.

3. Argyrol, when given by mouth or introduced into the trachea, does not lead to any reaction on the part of the endothelial phagocytes in the lung.

The writer desires to express his thanks to Dr. Rittinger who assisted in carrying out the animal experiments; also to Professor Klotz, Dr. Magner and Dr. W. L. Robinson for helpful suggestions.

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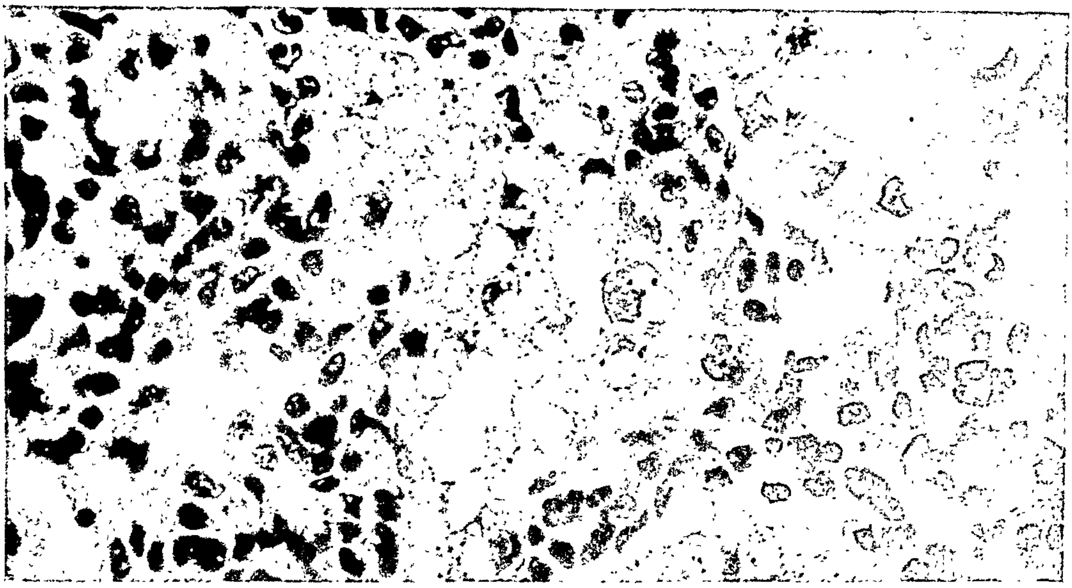
DESCRIPTION OF PLATE LXV

Fig. 1. This is a high power photograph showing oil phagocytes filling the alveoli. The outlines of many of the cells are indistinct but one large cell in the center of the field is clearly outlined and shows a rounded nucleus and a single nucleolus. This one contains two large droplets of oil. A few polymorphonuclear leucocytes are recognizable in the alveolar walls.

Fig. 2. Shows a number of oil phagocytes in the alveoli. They are not mixed with other cells and there is very little reaction in the alveolar walls.

Fig. 3. Shows many large typical oil phagocytes intermingled with red blood cells.



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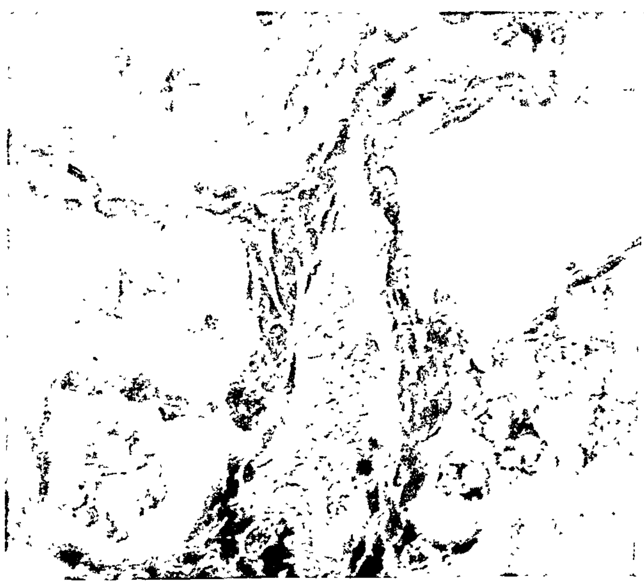

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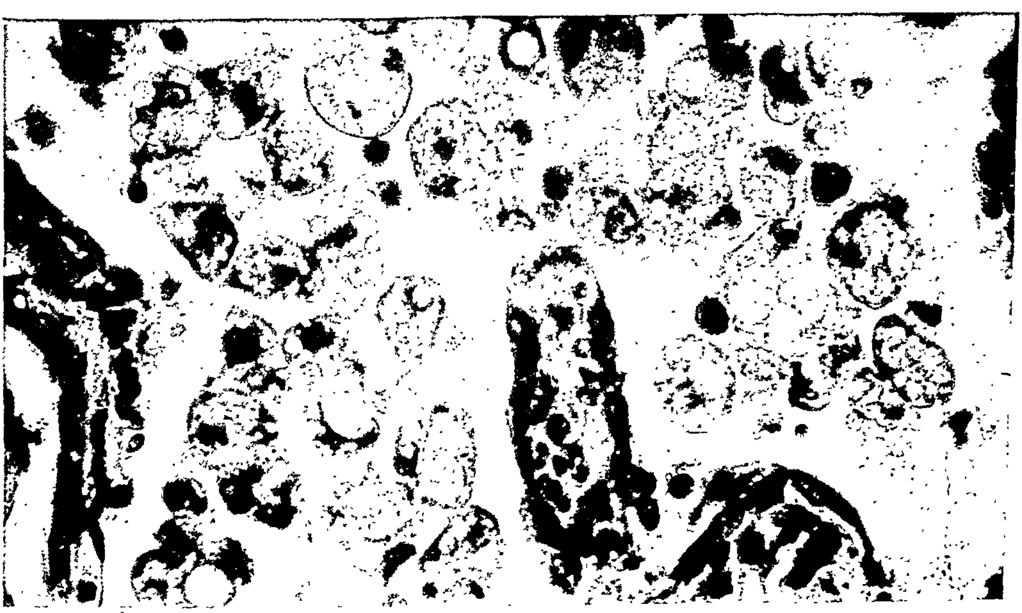

3

Laughlen Pneumonia following injection of oil

THE PATHOLOGY OF DIABETES, WITH SPECIAL REFERENCE TO PANCREATIC REGENERATION*

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But little opportunity has been afforded for the pathological study of cases of diabetes treated by insulin and for determination of what effects, if any, this treatment has had upon the lesions. Twenty-six autopsied cases of diabetes, the disease process varying in duration from two months to thirty years, form the basis of this report. These cases were under the care of Dr. E. P. Joslin, and the histories and clinical findings are unusually complete, with only one or two exceptions. Of these twenty-six cases, seventeen were treated with insulin over periods ranging from ten hours to thirteen months.

In order to present briefly the salient facts in the pathology of these cases, they have been combined in Table 1. Although statements as to the exact time of onset of diabetes are somewhat uncertain, it has seemed best to arrange the cases in order of increasing duration. One case, where the patient had been under observation twenty-six days, but had undoubtedly been suffering from diabetes much longer, has been placed at the end of the table.

Pancreas. Though the weight or estimated size of the pancreas is given in most cases, it is of little value in estimating the amount of pancreatic substance owing to the great variation in the connective tissue and fat, chiefly interlobular, present in the organ. Thus of two pancreases, both of which contained but little pancreatic tissue, one weighed 30 gms. and one 240 gms. This variation is not altogether dependent on the weight of the individual from which the organ came, as both women weighed over 200 pounds.

No attempt has been made to count the number of islands, owing to the inaccuracy of any feasible method. However, in six cases the islands appeared to be distinctly less numerous than in normal pancreases. No one distinctive lesion of the islands has been encountered in this series, as is to be expected in light of the work of Allen,[1]

* Received for publication May 11, 1925.

TABLE 1

Number	Age	Duration of disease	Pancreas: Wt. gms.	Pancreas: Islands	Pancreas: Acinar sclerosis	Heart: Wt.	Heart: Coronary arteries	Heart: Myocardium	Liver glycogen	Kidney: Glycogen	Kidney: Vascular disease	Aorta	Cause of death
4280	20.5	1 m. 27 d.	30	Lymphocytes	o	365	o	o	o	o	o	?	Septicemia
3502	64	6 m.+	116	H++	+	433	o	o	+	+	+	+	Multiple abscesses
2550	55	7 m.	?	H+++	o	?	S++	o	+	+	o	+++	Duodenal ulcer
3240	15.3	8 m.	Small	Few o	o	?	o	o	o	o	o	o	Coma
2720	10.2	11 m.	Small	Lymphocytes	o	?	o	o	+	+	o	o	Perinephric abscess
2446	36.9	1 yr. 1 m.	50	o	o	280	o	o	+	+	o	+++	Coma and pneumonia
1870	15	1 yr. 7 m.	Small	Lymphocytes	o	?	o	o	+	+	o	o	Coma
1907	24	1 yr. 10 m.+	Small	H++	++	?	o	o	?	+	o	o	Inanition
3210	65	1 yr. 10 m.+	Small	H+ few	++	250	S+++	S++	o	o	++	+++	Angina pectoris
2463	32.4	2 yr. 8 m.	Small	o	++	?	o	Hydrops	+	+	o	++	Abscesses of liver
4142	47.6	3 yr. 3 m.	80	H+++	+	290	o	o	+	+	++	+	Perinephric abscess
2988	56.3	4 yr.	Normal	H++	++	325	o	S+	o	+	+++	+	Pericarditis
1305	16	5 yr. 6 m.	Small	S+	++	?	o	S+	o	?	?	+	Coma
2470	69	9 yr.	Normal	o	++	325	S++	S++	+	+	++	+++	Chronic myocarditis
3670	46	9 yr. 6 m.	Small	Few S+++	+++	320	o	Hydrops S+	+	+	+	++	Appendicitis
896	69.2	10 yr. 8 m.	Cancer	H++	Cancer	330	S+++	S+++	+	+	+	++	Cancer of pancreas
3798	64	11 yr.	180	Few S+	++	315	S+++	S++	o	o	++	++	Gangrene and septicemia
705	66	14 yr.	?	H++	++	520	S+++	S+++	+	+	++	+++	Angina pectoris
1419	53.3	14 yr. 7 m.	240	S+H++	+	360	o	o	+	+	++	++	Appendicitis
263	48.7	14 yr. 9 m.	70	Few H+++	+	340	S+	S+++	o	o	++	++	Cardio-renal
870	53.2	16 yr. 11 m.	Normal	H+++		350	S+++	Aneurysm S+++	o	+	++	+++	Coronary thrombosis
3176	58.5	17 yr. 4 m.	Normal	S+++	+	?	o	S+	+	+	+	+	Phthisis
127	62	25 yr.	40	Few o	++	335	S+++	++	+	+	+	++	Coronary thrombosis
3242	52	25 yr.	?	H+++	++	?	+	o	o	o	o	?	Septicemia
1924*	53.9	30 yr. 2 m.	?	H++	+++	?	?	?	+	+	o	?	Gangrene and septicemia
3468	61	26 d. +	?	S+	++	610	S+++	S+++	o	+	++	+++	Gangrene and septicemia

* Restricted autopsy H = Hyalin S = Sclerosis ? = No data o = No pathology

Opie,[2] and Cecil.[3] Thirteen of the pancreases showed varying degrees of hyalinization of the islands. Only one of the thirteen patients whose islands showed hyalin change was under forty-five years old. Five showed more or less sclerosis of the islands, in three there was some lymphocytic infiltration about scattered islands, and there was no demonstrable pathology in the islands of five. The islands of three showed the type of enlargement described by Cecil [3] as adenomatous. In the few instances in which we found changes in the islands suggesting hydropic degeneration, post-mortem change could not be ruled out. The most likely case, No. 1870, only fifteen years old, died in coma after diabetes of short duration and was autopsied within an hour. No hydropic degeneration was apparent.

Through the kindness of Dr. Mallory we have been able to examine a slide of a pancreas sent to him by Dr. Willard S. Hastings from a case of fulminating diabetes. In this section every island shows extreme hydropic degeneration.

One outstanding feature of the various pathological changes is the wide variation in the condition of the islands in the same pancreases, and even in the same section. In one case of fourteen years' duration, No. 263, every stage is present from apparently normal islands to masses of hyalin imbedded in the stroma. We have found no pancreas in this series, no matter how severe the disease process or how marked the changes in the islands, in which a greater or less number of apparently normal islands could not be found. Moreover, a large number of pancreases from cases of diabetes other than those included in this series each showed at least a few apparently normal islands. Owing to the type of fixation of some of the tissues, Bensley's neutral gentian stain for the granules of island cells could be used only in a few cases. Through the courtesy of Mr. W. Bowie of Toronto we have been able to use his modification of Bensley's stain for the island cell granules in a number of cases where otherwise specific granule stains would have been impossible.

As regards the acinar tissue of the pancreas, varying degrees of sclerosis are present in some cases, independent of pathology in the islands. It is worthy of note that the acinar sclerosis is not apparent in any case much under two years' duration, even though the islands may show considerable change. In one case, No. 3592, which shows slight acinar sclerosis, the duration as given is six months, but an

accurate history could not be obtained and the disease probably had lasted for a much longer time. In another case, No. 896, most of the pancreas was replaced by a carcinoma and practically no acinar tissue was present. Islands persisted in the midst of the tumor and were very numerous in the tail, which had not been invaded by the tumor.

So far as we can determine there is no difference between the pancreases of those cases given insulin and those under dietary treatment alone, although we have the impression that in No. 263, treated with insulin for 13 months, there are more apparently normal islands than would ordinarily be the case.

There is no distinctive lesion in the young, uncomplicated cases of diabetes, as might be expected if there were one definite causal agent giving rise to the disease. Hyalinization of the islands occurs usually in those persons beyond middle age.

Heart. From this series, and from our experience with elderly diabetics in general, we feel that damage to the myocardium, chiefly through disease of the coronary arteries, is much more common in diabetics than in similar age groups from the general population. Of our seventeen patients over 40 years of age, three showed extensive healed infarcts in the wall of the left ventricle, one had an aneurysm of the wall of the left ventricle, five showed extreme sclerosis of myocardium (including two of the cases of infarct mentioned above), three showed moderate sclerosis (including the other case of infarct), four showed slight sclerosis and five showed none. The coronary arteries were correspondingly affected in these patients. In two cases there was occlusion of a main branch of the coronary artery, in one the left branch of the coronary artery was practically occluded, in seven there was marked coronary sclerosis, moderate sclerosis in two, slight in two, and no evidence of coronary disease in six. In two of the negative cases a single slide of the heart wall was the only tissue available.

Arteriosclerosis of the aorta generally ran fairly parallel with that of the coronary arteries, though in a few cases the aortic changes are either more or less marked than those in the coronaries. In one sixteen-year-old boy, No. 1305, who had very high blood fat, there were atheromatous plaques in the aorta and slight sclerosis of the myocardium.

We hope to make a detailed study of the heart and vessels in these and other diabetics, to be reported in a subsequent paper.

Liver. The only change characteristic of diabetes is the presence of glycogen within the nuclei of liver cells, and that is by no means pathognomonic of the condition. The liver cell nuclei show this deposit in fifteen of the cases.

The frequency of occurrence of gallstones in these cases is of interest, and is too great to be merely coincidence. Six cases, four of them males, totalling 30 per cent of those over thirty years old, showed gallstones at autopsy, and another case, also a male, had an obliterated gall bladder with numerous adhesions.

Kidneys. In the older cases there is a somewhat greater incidence of moderate chronic vascular nephritis than would be expected in a similar age group of non-diabetics, 78 per cent as against 69 per cent in a series of 100 autopsies, done in this laboratory, of non-diabetic patients over forty-five years old. This corresponds well with the amount of vascular disease. In six cases we could find no evidence of glycogen in the epithelial cells of Henle's loops.

TABLE 2

Insulin treated					Not insulin treated		
	Amount of insulin						
No.	Time	Units daily	Glycogen in liver	Glycogen in kidney	No.	Glycogen in liver	Glycogen in kidney
127	3 mos.	4–15	+	+	870	+	+
263	13 mos.	10–30	0	0	1870	+	+
705	6 days	2–6	+	+	1907	−	+
1305	11 weeks	2–16	0	−	1924	+	+
2446	1 day	100	+	+	2463	+	+
3176	8 days	15	+	+	2479	+	+
3240	10 hours	60	0	0	2559	+	+
3242	6 days	12–90	0	0	2720	+	+
3468	12 days	7–10	0	0	3210	0	0
3592	15 days	12–85	+	+			
3798	10 days	15–30	0	0			
4142	4 days	12–15	+	+			
3679	6 weeks	5–30	+	+			
2988	2 weeks	.....	0	+			
1419	1 day	50	+	+			
4289	22 days	5–75	0	0			
896	5 mos.	15–100	+	+			

Glycogen. In Table 2 is shown the occurrence of glycogen in the nuclei of liver cells and in the epithelial cells of Henle's loops.

Forty per cent of the insulin-treated cases show no glycogen, while it is absent in only 11.1 per cent of the cases under dietary treatment. This last percentage is rendered unduly high by the small number in the series; it is quite remarkable to find no trace of glycogen in Henle's loops in diabetics who have not had insulin treatment.

COMPLICATIONS

Gangrene. Seven of these cases, summarized in Table 3, developed gangrene. There appears to be a definite relation between gangrene and obesity.

These eight cases average 44 per cent overweight, as against 26 per cent for the cases which did not develop gangrene.

Acidosis. In all except one of the ten cases which had severe acidosis, but not coma, the condition was brought on by an acute

TABLE 3

No.	Gangrene		Arteriosclerosis	Maximum weight above standard %	Age
	Location	Duration			
127[1]	Left leg	20 days	Marked	55	62
1924	Right foot	4 days	———	66	54
2479	Left foot	14 weeks	Marked	40	69
3210	Left foot	2 weeks	Marked	11	65
3468	Right foot	10 days	Marked	46	61
3592	Right foot	15 days	Moderate	39	64
3798	Right foot	6 weeks	———	52	64

[1] Embolic in origin.

infection. That one exception was a young man of 24 whose diabetes was very severe. There is no apparent correlation between the condition and demonstrable pathology.

Coma. Of the six cases that died either in coma or within one month after recovery from coma, one omitted his insulin and had an infected hand, two broke diet, one developed pneumonia and another acute pericarditis, and one suffered from acute hyperthyroidism. Here again, as in acidosis, there is no characteristic pathological change. Four of the deaths occurred in those whose diabetes had lasted less than one year and seven months. None of these four

cases had demonstrable pancreatic pathology which could be clearly related to diabetes.

Out of 33 cases of coma reported by Joslin,[4] 17 occurred during the first year of the disease. That the first year of the disease is really the danger zone has long been apparent clinically [5] and generally explained as due to the patient's ignorance of means for its prevention or even to his ignorance of the existence of the disease. However, another factor must be considered; the regenerative power of the pancreas may be overwhelmed by the unknown toxic process at a time when the body metabolism is on a plane not yet adjusted to the disease. Especially in the young is this true for in them the metabolic requirements of growth add to the strain.

Obesity. There is a striking degree of obesity in most of these cases, particularly the older ones. The patients at their maximum averaged 32 per cent overweight, and if the five under 25 years of age are excluded, 37 per cent overweight. The weights given are for the most part those at onset of the disease. Unfortunately, the weight was not noted at autopsy in most cases. We feel, however, that the weight at death should be included in the autopsy data of all diabetic cases.

TABLE 4

Obesity in Relation to Heart and Vascular Pathology

Case No.	Per Cent Overweight	Pathology
3468	46	+++
3210	11	+++
2479	40	++
896	46	+++
3798	52	+++
705	29	+++
263	35	+++
127	55	+++
870	16	+++

+++ = extreme coronary sclerosis $\left\{\begin{matrix}\text{and}\\\text{or}\end{matrix}\right\}$ myocardial injury.

Duration. Several cases in this series were under observation for remarkably long periods. The lengths of time given are reckoned from the first definite diagnosis of the disease and so are minimal. The duration of the disease in seven is known for periods extending over fourteen to twenty-five years. One other, No. 1924, had been

refused life insurance repeatedly because of sugar in his urine thirty years before death. These clinically were relatively mild cases. In six of the eight cases the age at onset of the disease was under forty, and the average age at onset of all eight was thirty-six.

Of these eight cases, six showed a considerable degree of hyalinization of the islands of the pancreas, one showed marked sclerosis of the islands and extreme hyalin change of the arterioles of the pancreas, and the islands in another appeared normal, though seemingly decreased in number. In all the cases there was a greater or lesser degree of sclerosis of the acinar tissue.

The outstanding feature in all these cases is the presence of a few islands which show no demonstrable pathology, even though the majority may be seriously damaged.

Discussion

We have presented no direct evidence as to the etiology of diabetes, though obesity is very marked in some of these cases, especially in the older diabetics. The age at onset varies from ten to sixty years, and when above twenty years seems to have little effect on the duration of the disease so far as this series is concerned. The variation and inconstancy of the pancreatic lesions argue against an infectious origin for the disease, and the character of such lesions as may be present does not suggest the result of invasion by organisms. Indeed, one is tempted to wonder whether the changes in the islands may not be the result instead of the cause of diabetes. While it is true that in a few relatively early cases of diabetes we found lymphocytes about the islands, this can hardly be considered as evidence for an infectious origin of the lesion.

Whatever the cause may be, it seemingly acts over a long period of time, perhaps throughout the duration of the disease. The pathology which we find in the pancreas at autopsy rarely represents the initial damage to the organ, but rather the result of a long struggle between the regenerative activity of the pancreas and the degenerative changes caused by the diabetogenic factor. The pancreas is not a static organ like the brain or myocardium, unable to repair itself after injury.

For some reason this static conception of the pancreas has become firmly established in spite of clinical and anatomical evidence to the contrary, probably because the diabetic patient cannot be cured and

frequently goes steadily downhill in spite of treatment. We believe that this unfavorable course of the disease is not due to failure of the pancreas to regenerate, but to continued injurious action on the organ by the causal agent, eventually overcoming the regenerative efforts. In a subsequent paper we are presenting the clinical evidence given by this same series of patients.

Perhaps one reason for the skepticism as to pancreatic regeneration in diabetes is the lack of the classical evidence of such activity — cells in mitosis and cells in abnormal situations and relations with one another.

We have found evidences of the power of the pancreas and of the island tissue in particular to regenerate after acute injury. Thus in a pancreas from a non-diabetic patient dying of lobar pneumonia (A15–16) we found numerous mitotic figures in the island cells, as high as seven mitoses in a single island (Fig. 1). Mitotic figures can occasionally be found in the island cells of cases dying of diphtheria and of lobar pneumonia. At times necrotic cells are found. This injury and subsequent repair not only indicate the regenerative power of the pancreas, but perhaps explain the transient glycosuria occasionally encountered in acute infections.

This same transient injury to the islands may explain the severe drop in sugar tolerance noted in diabetic patients during acute infections. The rapid reëstablishment of the former level of sugar tolerance following recovery from the acute process may well represent the result of the rapid regeneration of the island cells.

Bensley [6] states that in experimental animals after duct ligation occasionally a pancreas will be found which differs from the normal only by smaller size and increased fibrous interstitial tissue. This is interpreted as due to regeneration following accidental reëstablishment of the connection between duct and bowel. Bensley believes that under appropriate conditions the capacity of the pancreas for regeneration is 100 per cent.

Boyd and Robinson [7] have reported a case of a nine-year-old diabetic boy, accidentally killed after six months of insulin treatment, whose pancreas showed definite evidences of regeneration and whose insulin requirement had steadily fallen.

In one of our cases, No. 896, whose diabetes had lasted for fourteen years and who had been under insulin treatment for five months, receiving 15 to 100 units daily with gradual decrease of the

insulin requirement, there is evidence of regeneration of the islands. No acinar tissue is present. The bulk of the pancreas is occupied by a carcinoma, from which the patient died, but the tail is not invaded by the tumor. Here the islands are closely packed. Some show a moderate degree of hyalinization, but most are entirely free from hyalin or other degenerative changes. Columns of cells extend out from the islands into the surrounding stroma, and in places entire low power fields are made up of island tissue. There are more islands than could be accounted for by their concentration by contraction of the stroma of the pancreas following destruction of the acinar tissue.

In any disease as insidious in onset and as chronic as diabetes, with pathological changes largely restricted to one portion of a single organ, one cannot expect any striking evidences of either destruction or regeneration. It is not unnatural that the conception of the diabetic pancreas as an inert organ, passively submitting to gradual destruction, has become firmly established.

We may assume that the lesions in diabetes are not infectious but toxic in origin. Their course is extremely chronic, and consequently the attempts at regeneration are slow. Mitotic figures would hardly be expected under the circumstances.

Practically all toxic lesions of the same age in the same organ resemble one another, as in toxic myocarditis or central necrosis of the liver. But in the islands of the pancreas showing either hyalinization or sclerosis, practically every stage from masses of hyalin or dense connective tissue imbedded in the stroma to apparently normal islands can be found. It is difficult to conceive a toxic substance of very chronic action or a long-continued functional strain totally destroying one island and completely sparing the next. Much more logical is the assumption that we are dealing with a gradual destruction of islands, a formation of new islands to replace them, exposure of these to the toxic substance with consequent pathological change and still more islands formed to take their places. The apparently normal cells found represent those most recently formed. Eventually the destructive process wears down the regenerative powers of the organ and the end comes.

Hemochromatosis, often known as bronzed diabetes, gives us an excellent opportunity to test this assumption. In this disease we are dealing with a known injurious agent, hemofuscin. This is a break-

down product derived from hemoglobin and is deposited in various cells of the body, where it very slowly changes to hemosiderin. Eventually the accumulated pigment causes necrosis of the cell containing it. The liver is the first site of deposit, but as its cells become filled, the pigment overflows, one might say, to other organs. The pancreas is one of these.

In those cases of hemochromatosis where the pancreas has become seriously involved before death, diabetes occurs. If the pathology referable to the pigment cirrhosis of other organs be disregarded, this diabetes differs in no whit from diabetes mellitus, except that the course is more rapid.

Here then we have an ideal means of studying diabetes, with a known etiology and a fairly rapid course.

We have had the opportunity of studying several cases of bronzed diabetes in this laboratory. We find the same variation in involvement of the islands as we have mentioned in our cases of diabetes mellitus, ranging from the remains of islands represented by clusters of pigment-loaded endothelial leucocytes and fibroblasts in the stroma to islands without pigment and apparently normal. The conclusion is inevitable that new islands are being formed to take the place of those destroyed by the pigment deposits. In further substantiation of this evidence, occasional mitotic figures (in Case A 17–8) can be found in the cells of the younger, pigment-free islands (Figs. 2, 3).

The pigment is not restricted to the island cells, but affects the acinar tissue as well. The same evidence of regeneration is offered by the acinar cells as by those of the islands.

The well-established evidence of destruction and regeneration of parenchymal cells in the liver offers a striking parallel to the changes in the pancreas in hemochromatosis. Just as in the liver the parenchymal cells show every stage from newly-formed pigment-free cells through those containing hemofuscin and those containing hemosiderin to necrotic cells, the same steps can be traced in the acinar and island cells of the pancreas.

If we substitute diabetes mellitus for hemochromatosis, hyalin formation in the islands for pigment deposit in the island cells, the analogy is complete.

There is no reason to doubt that the increased fibrous tissue noted in the pancreas in some cases of diabetes mellitus accumulates in the

same way as the fibrous tissue in cirrhosis of the liver. The parenchymal cells, sometimes of the islands, sometimes of the acinar tissue, or of both, are killed and disappear. Their stroma remains behind. The parenchymal cells regenerate and new stroma forms to support them. In this way the fibrous tissue gradually increases in amount. The increased fibrous tissue noted in the pancreas in some cases of diabetes is therefore not due to a simple proliferation of the interacinar and interlobular connective tissue. Probably in most cases there has been damage to and regeneration of the acinar tissue as well as the islands. There is some clinical evidence of disturbed external secretion of the pancreas in diabetic patients.[8]

In our series fibrosis of the pancreas is not found in those cases whose diabetes had existed less than two years. However, some of the cases of fairly long duration do not show any great increase in fibrous tissue.

Aside from the pathology of the pancreas, the changes in the blood vessels and myocardium are of interest. The frequency of severe myocardial damage and of sclerosis of the coronary arteries and the aorta is much greater than would be expected for similar age groups of non-diabetics. Of course our series is too small from which to draw any definite conclusions, but it does indicate an abnormal prevalence of vascular disease among diabetic patients. This may well be related to the abnormal fat metabolism and the striking tendency toward obesity.

In one sixteen-year-old boy (No. 1305) there were found at autopsy atheromatous plaques on the aorta. He had a high blood fat, and it seems quite possible that this is related to the arteriosclerosis. In addition, large numbers of lipoid-filled cells are present in the spleen. A similar case, a man of 22, reported by Smith,[9] showed slight atheromatous plaques in the aorta.

The frequency of chronic vascular nephritis in our series is also somewhat higher than is encountered in non-diabetic patients, reënforcing the other evidences of vascular disease.

So far as differences in pathological findings in those cases treated with insulin and those not treated with insulin are concerned, there is nothing startling. We have an impression that there are rather more normal appearing islands in the pancreases of insulin-treated cases than in those of cases under dietary treatment alone. Glycogen is much less frequently found in the liver cell nuclei and in the

cells of Henle's loops in insulin-treated cases, as might be expected with the improved utilization of carbohydrates.

Summary

1. The pathological findings in twenty-six cases of diabetes mellitus are presented. The duration of the disease in eight of these is known to be over fourteen years.

2. Thirteen cases show hyalinization of the islands of Langerhans. Only one of these cases was under forty-five years old. Five show varying degrees of sclerosis of the islands. In three more there is slight lymphocytic infiltration about scattered islands, and the islands in five appear normal, though decreased in number in two of these five.

3. Apparently normal islands are present in all pancreases examined, no matter how badly damaged the bulk of the islands may be. In two cases, insulin-treated over five months, there seem to be more islands of normal appearance than in cases under dietary treatment.

4. The character of the lesions in the islands of Langerhans suggests a toxic origin, and an injurious agent acting over a long period of time.

5. Occasionally in acute infections, such as lobar pneumonia and diphtheria, there is toxic injury of the island cells, and subsequent regeneration of the islands. This is probably the explanation of the transient glycosuria sometimes met with in the course of acute infections.

6. In hemochromatosis there is definite evidence of regeneration of both the acinar tissue and island tissue of the pancreas. The type of diabetes in hemochromatosis is the same as that in diabetes mellitus.

7. The increased fibrous tissue found in certain pancreases in diabetes is due to destruction of island or acinar cells or both, with persistence and condensation of their stroma. The parenchymal tissue regenerates and forms new stroma, with resulting increase in connective tissue.

8. The acinar tissue of the pancreas does not show evidence of increased fibrosis in cases of diabetes of short duration.

9. Insulin treatment decreases the frequency with which glycogen is found in liver cell nuclei and in the epithelium of Henle's loops.

10. The frequency of severe myocardial damage, due to coronary sclerosis, is high in diabetics over forty.

11. The patients developing gangrene averaged 44 per cent overweight, while the others averaged 26 per cent overweight.

12. The patients over twenty-five years of age in this series averaged 37 per cent overweight.

Conclusions

1. A new interpretation is offered of the pathology of the pancreas in diabetes mellitus.

2. The long-continued action of an injurious agent (or possibly excessive functional activity) causes a gradual destruction of island, and at times of acinar cells. New cells are formed to take the place of those destroyed, only to be exposed to the injurious influence with consequent pathological change. Their injury is followed by the production of still more new cells.

3. Eventually the destructive process wears down the regenerative powers of the pancreas, thus explaining the unfavorable course of the disease.

4. In pneumonia and other infectious diseases, the pancreas readily regenerates after acute injury.

5. The disturbed carbohydrate metabolism giving rise to abnormal fat or protein metabolites may be the cause of the high incidence of vascular disease in diabetic patients.

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DESCRIPTION OF PLATE LXVI

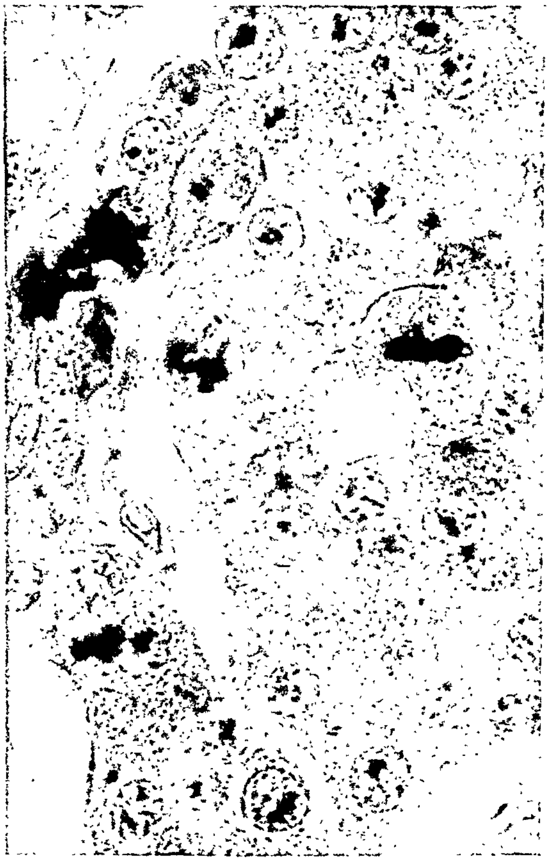
Fig. 1. Island of Langerhans from case dying of lobar pneumonia, showing three mitoses. x 1000.

Fig. 2. Island of Langerhans from case dying of hemochromatosis, showing pigment-free island and two mitotic figures. x 1000.

Fig. 3. Island of Langerhans from case dying of hemochromatosis, showing one mitotic figure, and small amount of pigment. x 1000.

Fig. 4. Island of Langerhans from case dying of hemochromatosis, showing one mitotic figure.

Photomicrographs by Dr. F. B. Mallory.



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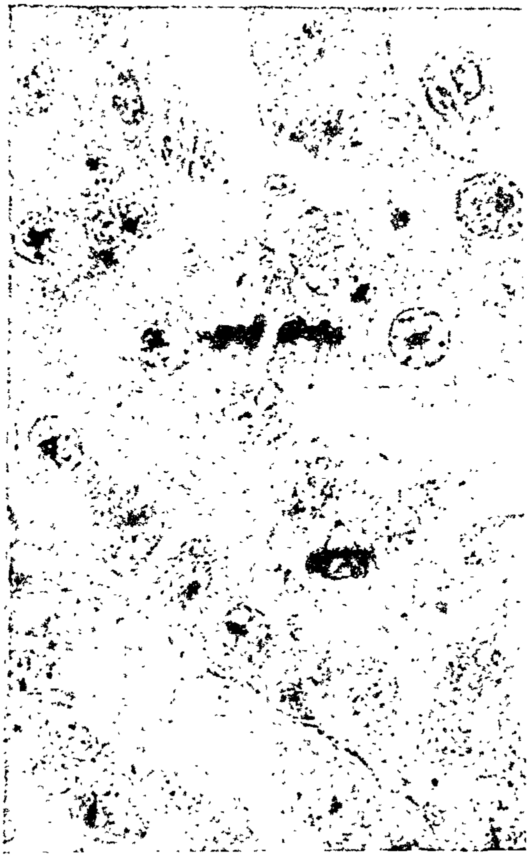

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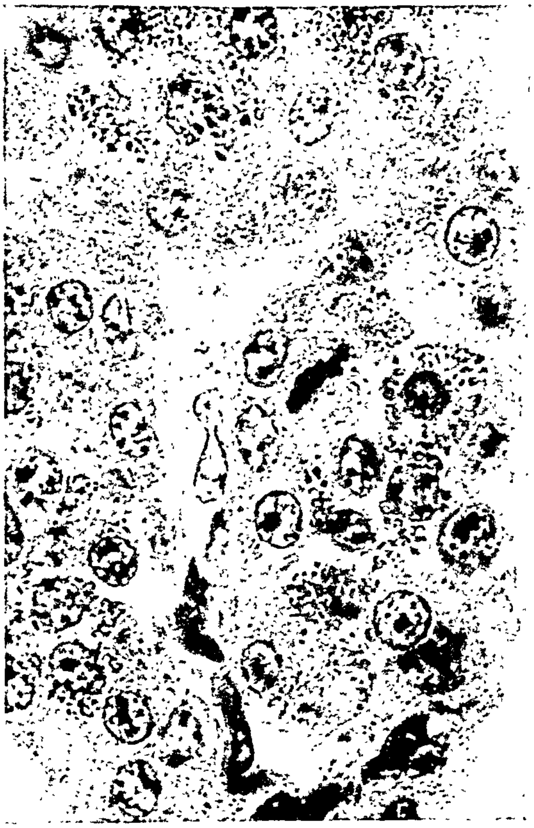

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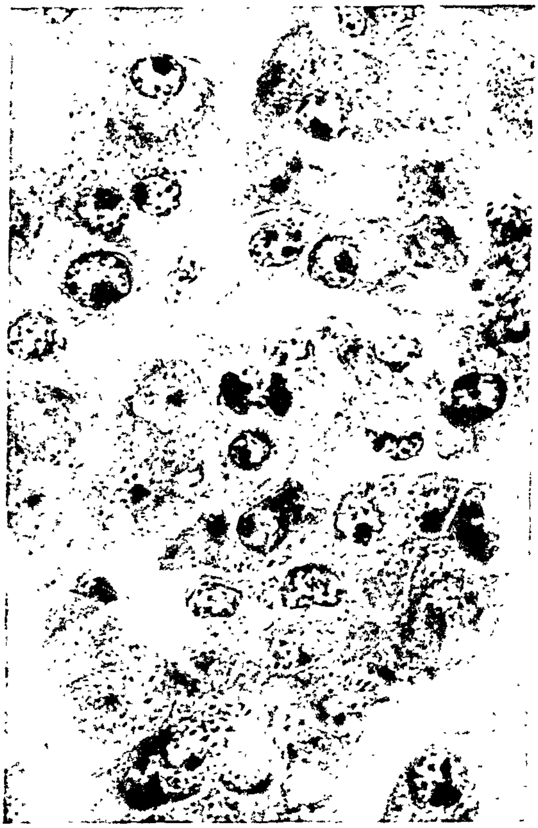

4

Warren and Root The pathology of diabetes

THE OCCURRENCE OF RETICULUM IN TUMORS *

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Introduction. Since von Kupffer discovered the "Gitterfasern" of the liver in 1876, and Mall (1891, 1896) first distinguished the reticular connective tissue fibers from the collagenous fibers, reticulum has been the subject of considerable investigation; its occurrence in various organs under conditions of health and disease, its relationship to collagenous connective tissue, and its histogenesis (which is still in doubt) have all been studied. The reader may find an extensive bibliography in Russakoff's (1908) article on the pulmonary reticulum. At the time he wrote, the introduction of the Bielschowsky-Maresch silver impregnation technic had given impetus to the investigation of reticulum; Kon (1908) had studied it in the liver, Rössle and Yoshida (1909) in the lymphnodes, while Russakoff examined sections of liver, kidney, pancreas, and lymphnodes as well as lung. Neuber (1912) investigated the cardiac reticulum in health and disease, and more recently Corner (1920) and Miller (1923) have attacked the problem as it applies to the capillaries and the lung.

A survey of this work would lead the reader to conclude that reticulum is the product of cellular activity, that it may be connected with the blood-vessels on the one hand, or serve as a supporting, reticulated membrane for epithelium on the other. It forms practically the sole supporting tissue in lymphnodes. That it is the product of endothelial cells and, occasionally, fibroblasts, seems to be the general belief; some authorities claim the reticuloendothelium, some the vascular endothelium as well, as its progenitor.

One may readily study the distribution of the reticulum in various organs by means of silver impregnations according to Bielschowsky's technic, so that further preliminary discussion of this subject is unnecessary; let it suffice that it is very widely distributed and its

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importance is often overlooked. For example, its widespread occurrence in epithelial organs, where it forms a dense basement membrane, is insufficiently recognized. That it may with time, and under rather uncertain conditions, become transformed into collagenous fibrous tissue is becoming more universally believed. Russakoff, Rössle and Yoshida, and Miller have all stressed this point. That it is the product of cellular activity on the part of endothelial derivatives is, however, becoming a debatable question. Miller has pointed out the close relationship between newly-formed reticulin fibers and neighboring, preëxisting reticulum; Baitsell (1915, 1916) has advanced anew the theory of purely extracellular origin; and one of us (Foot, 1925) has recently stated his belief that we must look elsewhere than to cells for the origin of reticulin fibrils, basing this view on observations on the development of reticulum in experimental tubercles.

As nothing appears to have been done in connection with the distribution and occurrence of reticulum in tumors, a hundred or more have been collected in the past three years' accumulation of laboratory material of this department, and these have been studied with special reference to their reticulum content.

Technic

In most cases Zenker-fixed material is available, but where it is not, formalin-fixed tissue has been used. Several serial sections were cut from each paraffin block, three being stained with the routine Harris' hematoxylin-eosin stain and two by a modification of the Bielschowsky-Maresch technic devised by one of us last year. (Foot, 1924.)

Briefly summarized, the steps are as follows. Sections about five microns in thickness are cut in paraffin. Zenker fixation is superior to formalin, but the technic is the same in either case. After removing the paraffin, treat five minutes with a weak alcoholic solution of iodine, removing this by a brief immersion in 5 per cent "hypo." Wash and treat for five minutes with 0.25 per cent potassium permanganate in water. Wash and place the sections in 5 per cent aqueous oxalic acid. Wash in distilled water and impregnate for forty-eight hours with a 2 per cent solution of silver nitrate in distilled water. Wash in distilled water and treat with Bielschowsky's silver-ammonium oxide solution for one half-hour. Rinse in distilled

water and leave in 5 per cent formalin (2 per cent formaldehyde) for another half-hour and then, after washing in tap-water, tone the sections for one hour in a one per cent aqueous solution of gold chloride, after which a two-minute immersion in 5 per cent "hypo" will remove any superfluity of the silver. The sections may be mounted at this point, but it is better to counterstain for the usual time in Harris' hematoxylin, wash, and stain for forty-five seconds in Van Gieson's picric-acid-acid-fuchsin. Run the sections immediately through ascending percentages of alcohol into xylol and mount in balsam, avoiding water as it decolorizes the fuchsin. After the silver impregnation the sections should be practically colorless, turning brownish-black in the formalin and fading to gray in the gold bath. The "hypo" turns them old-rose and gray. The finished, counterstained sections show the reticulum in sharp black lines, the collagen in vermilion to crimson. The nuclei are brownish, the cytoplasm and muscle substance yellow. The silver impregnation is performed in subdued daylight, not in the dark.

Reticulum Fibers in Tumors

Epithelial Tumors

Surface epithelium. The epidermoid carcinoma is of paramount interest here, the benign epithelial tumors of this type conforming closely to normal histology. In these carcinomata there is a varying amount of reticulum; plentiful in the corium, it forms a network of delicate fibrils about the advancing columns of tumor cells which dip down from the epidermis. Generally speaking, if the tumor be of rapid growth reticulum is plentiful, if slowly growing more collagen than reticulum develops. Usually the latter forms a membrane-like plexus at the base of the epithelial plugs, occasionally it penetrates them superficially. A metastasis from such a tumor is shown in Fig. 1. In the basal cell, or hair-matrix carcinoma the reticulum forms a delicate basket-work around the cell masses, with radiating fibrils that are continuous with those of the corium.

Glandular epithelium. The common adenoma usually shows more collagen than reticulum in its stroma, the various types of fibro-adenoma following this rule, but a narrow zone of reticulum is commonly found about the base of the acini, outlining them in black. In a rapidly growing papillary adenoma the stroma is composed al-

most entirely of reticulin fibrils, to the comparative exclusion of collagen. If the tumor acini are closely compressed one observes reticulin fibrils between them; lying almost, if not quite isolated and free from any great admixture of mesenchymal cells. Capillaries carry a sheath of reticulum, but frequently there are but few vessels in this stroma and the reticulum appears to have been produced independently of cellular agency.

The various types of adenocarcinoma and medullary carcinoma all follow the description just given; the cell-masses in the medullary type are usually quite free from penetration by the reticulum, but very rapidly growing tumors, with a tendency to cell dissociation, may show a good deal. Fig. 2 shows a typical adenocarcinoma. If the tumors have metastasized to lymphoid tissue the lymphoid reticulum will, naturally, be found intimately intermingled with their cells. In the colloid type, those cells which remain at the center of the mucoid masses may have small, woolly plexuses of what seems to be reticulum about them, but the production of fibrils resembling reticulin is quite common in necrotic areas in epithelial tumors and it is doubtful if such fibrils constitute true reticulum. Scirrhous carcinoma, as one might expect from its slow, sclerotic growth, shows large amounts of collagen in its stroma and a very variable quantity of reticulum, which is usually scanty. The sarcomatoid type of pancreatic carcinoma, shown in Fig. 3, presents a moderate amount of reticulum in its stroma and there is some penetration of this tissue into the more or less dissociated groups of tumor cells, which is also true of the similar type of carcinoma of the thyroid. The difference in the distribution of reticulum in these tumors and that in lymphosarcomata is, however, so striking as to render a diagnosis comparatively simple in cases that would be quite obscure when stained in the usual way.

Hypernephroma, or adrenal carcinoma, shows a very small amount of reticulum which does not penetrate into the alveolar cell masses. Although this tumor is found in the kidney, an organ rich in reticulum, there is practically none in the alveoli which push the preëxisting fibers aside.

The tumor section photographed for Fig. 4 was kindly sent to us from the Albany Hospital, by Dr. Victor Jacobson, and is worthy of special mention. It is from a medullary carcinoma of the uterus, which differs in no way from any other such carcinoma excepting

that its stroma shows an extraordinary anaplasia to what appears to be a true fibrosarcoma. Thus two malignant tumors are intimately combined in one section. The carcinoma is practically free from reticulum while its stroma shows an abundance of straight, coarse reticulin fibrils exactly analogous to those of the fibrosarcomata to be discussed presently.

Endothelial Tumors

Vascular. Cavernous hemangiomata show a reticulum about their vessels and sinuses quite similar to that normally seen surrounding capillaries. A malignant perithelioma appears to produce very little reticulum, as this is found only in the immediate proximity of the vessels. As we could secure only one specimen, this negative finding should be confirmed.

Reticuloendothelial. Several types of malignant endothelioma were examined, some showing comparatively little reticulum, some a great deal. Of the latter group, the primary lymphnode endothelioma, so closely resembling medullary carcinoma, is a good example. Where the carcinoma shows little reticulum in its alveoli, the fibers usually stopping short at their margin, or simply traversing them in straight, unbranching lines, the endothelioma exhibits an abundant, branching plexus of curving reticulin fibrils intimately associated with the cells of the tumor within its alveoli. The fibrils are distinctly plexiform and anastomotic in this case, which affords a good criterion for the diagnosis of doubtful alveolar tumors of the axillary lymphnodes in women, where primary endothelioma is readily mistaken for the more common metastatic alveolar carcinoma of the breast. We feel this to be the chief contribution of this paper. (Figs. 5 and 6.)

Another type of endothelioma, supposedly originating in the reticuloendothelium of lymphnodes, is composed of polygonal, anastomosing cells, and shows an abundant reticulum intimately related to these cells and following their cytoplasmic processes very closely. A given fibril may, however, course over several cells without any interruption in its substance and the unbroken continuity of these fibrils with those of the neighboring normal tissue is always striking. This tumor (Fig. 7), was recently reported by one of us (Foot, 1924) in detail.

The endotheliomata arising in bone-marrow correspond in the

main with the loosely reticulated portions of the primary endothelioma of lymphnodes, insofar as their reticulum is concerned; but they contain somewhat less and the fibers are far more delicate. One case of diffusely disseminated cells, resembling endothelial phagocytes and occasionally grouped into tumor masses, shows marked reticulum production wherever the cells become so massed.

Dural endothelioma. This tumor (Fig. 8) probably produces more reticulum than any other tumor we have observed; there is a dense felting of reticulin fibrils in its matrix and they are intimately intermingled with an almost equally dense mass of collagen fibrils.

Mesothelioma

A tumor from the pleural cavity, connected with the lung, is composed of squamous cells that tend to keratinize and to form pearls. It resembles an epidermoid carcinoma in every respect, but there is little upon which to base this diagnosis if one considers that the tumor lay in and beneath the visceral pleura, not near large bronchi, and there was no history to suggest bronchiectasia. A similar growth is described by Beitzke, in Aschoff's "Pathologische Anatomie" (1923), as a "pleural cancer." We prefer the term "mesothelioma" and feel security in our diagnosis for the reasons just given. This tumor shows an abundant reticulum between its cell masses and a moderate amount of collagen; it is almost exactly like an epidermoid carcinoma in respect to its stroma. Its cell masses are entirely free from reticulin fibrils.

Fibroblastic Tumors

Fibroma. Both types of fibroma, the soft and hard, are chiefly collagenous; practically no reticulum is seen, save around the blood vessels. (Fig. 9.) A very mucoid fibroma of the external auditory canal, which recurred twice in a year, shows rather scanty collagenous tissue with wide, unstained spaces and an abundance of short, rather straight reticulin fibrils. (Fig. 10.) It is not a true myxoma, for there is no bluish hyaline-matrix, and it is not malignant. Here there appears to be a direct causal relationship between the presence of reticulum and rapidity of growth.

Keloid. Both the keloid and the fibrous granuloma show reticulum and collagen in about the same proportions as occur in normal corium; the total amount of these fibers, however, is greatly in-

creased. The keloid has rather more collagen than reticulum, however.

Giant cell tumors. These are not rich in reticulum and show only moderate amounts of collagen. The giant cells become deeply stippled with silver, as is the case with some endothelial cells and with nerve cells. The tumors are chiefly cellular.

Fibrosarcoma. Two types are recognizable: one producing almost nothing but reticular matrix (Fig. 11), and one an abundance of collagen (Fig. 12). The former is very similar to the reticulo-endothelioma with branching cells, already described. Apparent transitions between the reticular and collagenous types are seen, but the degree of anaplasia and the rate of growth will not entirely explain this difference — for one very anaplastic and rapidly growing fibrosarcoma of the lung shows little or no reticulum, but produces an abundant collagenous matrix (Fig. 13). Generally speaking, however, there is more reticulum in the rapidly growing sarcomata and more collagen in those of slower development.

It is interesting to compare the tumors in our series of fibrosarcoma as regards their reticulum content. One, a sarcoma of the scapula (Fig. 14) of rapid growth, shows an evenly distributed mesh of very fine, short, and curly reticulin fibrils, many of them beaded and interrupted. This indicates very young reticulum, judging from the literature and from personal observation on its formation in tubercles. Very little collagen is present. Then come a sarcoma of the lung, one of the kidney, and a third from the bladder, all of which show coarse, straight reticulin fibers and little collagen. At the other end of the scale we find a sarcoma of the lung and one of the knee, both of which show much collagen and little reticulum. From this one may infer that reticulum is laid down by the more embryonal type of rapidly growing tumor and that it may ultimately be changed into collagen. The very primitive fibrosarcomata and reticuloendotheliomata both revert to a common type, the mesenchymal. Hence their similarity and the comparative uselessness of attempting to classify them by adult-cell standards, or to distinguish them apart.

Other Connective Tissue Tumors

Lipoma. Tumors of this type produce more collagen than reticulum, as one would expect after a survey of normal fat.

Liposarcoma. In one case we find short, wavy, more or less beaded reticulin fibrils and little collagen. (Fig. 15.) In the others, which resemble Fleming's fat organ, there is abundant reticulum and but little collagen.

Leiomyoma. Here much collagen is produced; often more of this is seen than of muscular tissue and a large amount of reticulum closely invests the muscle fibers. Is this reticulum converted into the abundant collagen that gives the clinical name of "fibroid" to the tumor?

Leiomyosarcoma. This shows an abundance of both reticulum and collagen, the latter in the form of short fibers and not at all as profuse as in its benign prototype. Here again we see more reticulum in the more youthful form of tumor.

Rhabdomyosarcoma. One of these, from the endometrium of an old woman, was examined. It is seen to be composed of alveolar septa of collagen, and in the spaces thus formed is a very loosely made network of reticulin fibrils, sometimes short and curly, sometimes nearly straight and much longer, but always wavy in contour. In the smaller spaces between these fibrils lie the rhabdomyoblasts, usually quite isolated. An abundance of cells resembling fibroblasts are also present and the reticulum is more apt to adhere to these than to the muscle cells.

Melanosarcoma. Melanosarcomata are abundantly supplied with collagen, but a good deal of reticulum is found penetrating the alveolar cell masses and running among them almost as freely as in the primary endothelioma of lymphnodes.

Chondroma and chondrosarcoma. Benign chondroma shows practically no reticulum, while chondrosarcoma shows a great deal in its more immature portions, the production of chondromucin later obscuring it. Sometimes the reticulum persists in this matrix and its fibers may be seen through the homogeneous chondromucin.

Osteoma. Aside from the marrow spaces, no reticulum is seen in this form of tumor. We did not obtain an osteosarcoma and cannot report on its reticulum.

Lymphoid Tumors

Lymphoma and Lymphosarcoma. Here one finds practically no fibers other than reticulum, and this may be quite unassociated with any cells resembling reticuloendothelium; in one malignant lym-

phoma of the small celled type the reticulum lies quite free in a mass of dissociated microlymphocytes, and one can see no connection between it and any other type of cell. It appears to have been laid down by some process independent of cellular activity, or borrowed from the lymphoid tissue.

The commoner lymphosarcoma, with its larger type cell, exhibits an abundant reticulum that branches all through the tumor and is in unbroken continuity with that of the lymphnode. (Fig. 16.) Where the tumor penetrates the capsule and invades the surrounding tissue, it forms a new reticulum that is continuous with the older.

Hodgkins' granuloma. The amount of reticulum produced in the Hodgkins node varies a great deal; sometimes there is much, at others (Fig. 17) there is practically no new reticulum to be found.

Tumors of the Nervous System

Glioma. This shows no reticulum other than that of its vascular supply.

Neuroblastoma and Ganglioneuroma. The same is true in this case, which makes it possible to differentiate these neoplasms from other types which might be confused with them. Neuroblastoma often resembles lymphosarcoma in routine sections, but a reticulum stain will readily differentiate the two.

Amputation neuroma and neurofibroma. There is very little reticulum in these, and it is confined to the connective tissue of the epineurium and its prolongations. In these there is much collagenous tissue.

Pituitary struma. One chromophile struma was examined, from a case of well-marked acromegaly. This tumor is so dissociated in its structure that practically the only stroma present is seen in the immediate vicinity of its vessels, in the form of perivascular reticulum.

Mixed Tumors

Mixed tumor of parotid. Several of these were studied, all showing a variable amount of reticulum about the epithelial islands and ducts. Collagen varies, being most abundant in the more mixed, and therefore more mature varieties; with cartilage and heavy connective tissue septa. The more youthful, epithelial type of tumor shows practically no collagen and a very dense reticulum about its ducts and acini. (Fig. 18.)

Teratoma. Several of these, one a malignant embryoma with rather advanced differentiation, were examined. No rule can be laid down for such diversified tumors; if they contain those types of tissue normally rich in reticulum, the latter will naturally be present.

Summary

From what we have said, it is evident that reticulum is a common and widely distributed constituent of the stroma and matrix of tumors, the only marked exceptions being those of the nervous system. Some of the very fibrous tumors show so much collagen that the reticulum is overshadowed and may, indeed, have been replaced by this tissue. In general, there appears to be a definite connection between rapidity of growth and reticulum formation, for this tissue is more abundant in young and rapidly growing tumors than in those of more adult type and slower progress. It is indicated that this would be converted into collagen in time; that this is not an invariable rule is shown by the presence of much collagen in some rapidly growing sarcomata. That anaplasia is not necessarily connected with the production of reticulum is indicated by the coincidence of reticulum and good differentiation in a benign fibroma, several lipomata, and other mature tumors and by its absence in tumors showing marked anaplasia.

One point that cannot be stressed too emphatically is our failure to find any definite relationship between cellular structures and reticulum. Although it is usually near vessels and is undoubtedly more abundant in their vicinity, it often appears to be quite unassociated with cytoplasm. It is often as abundant in the proximity of epithelial cell masses as it is near endothelial or fibroblastic cells, although it is undeniably more abundant in the case of endothelial tumors. It seems that the conditions for its production are more favorable in these tumors but that nothing points directly to the cellular origin of reticulin fibrils. As in the case of tubercle reticulum, there appears to be a closer relationship between reticulum and preëxisting reticulum than there is between that tissue and any cells. It is evident that we must keep our minds unclouded by dogma and further test out the hypothesis of the intercellular, or extracellular, origin of reticulum. The production of these fibrils may prove to be more or less similar to that of fibrin, a process of precipitation and accretion, rather than one of intracellular differentiation.

Conclusions

1. Reticulum is a regular constituent of the stroma of most tumors, excepting those of the nervous system.
2. It is usually most abundant in tumors of rapid growth.
3. It is apparently converted into collagen in the more slowly growing neoplasms.
4. It does not show any constant relationship to cellular constituents of tumors, but seems to be laid down in continuity with preëxisting reticulum in the intercellular fluids or substances by a process independent of cytoplasmic differentiation and analogous to precipitation or crystallization.
5. Silver impregnation of tumor reticulum constitutes a valuable diagnostic method, especially in the case of tumors of endothelial, lymphoid, and nervous-tissue origin.

This work represents the examination of 135 tumors, of which: 23 were adenocarcinoma, 11 fibrosarcoma, 10 lymphosarcoma, 9 epidermoid carcinoma, 8 endothelioma of various types, 7 fibroma, 4 leiomyoma, 4 lipoma, 4 Hodgkins' nodes, and 4 melanosarcoma. The rest were represented by one or two examples, either because it was obvious that they were typical, or because of lack of material, as in the case of neuroblastoma, ganglioneuroma, and rhabdomyosarcoma.

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DESCRIPTION OF PLATES LXVII–LXXI

Plate LXVII

Fig. 1. Metastasis from an epidermoid carcinoma of the penis. The epithelial alveoli are not invaded by reticulum, which merely outlines them.

Fig. 2. Medullary form of adenocarcinoma primary in stomach. Here there is more dissociation of the cells and consequently slight invasion of cell masses by reticulum.

Fig. 3. The sarcomatoid type of pancreatic carcinoma. There is comparatively little reticulum, which tends to invade cell aggregations. Cf. with lymphosarcoma photomicrograph.

Fig. 4. Medullary carcinoma of uterus, with sarcomatous stroma. Specimen of Dr. Jacobson's. Note the very heavy, coarse reticulum of the stroma, and compare with that of the preceding figures and with that of the fibrosarcomata.

Plate LXVIII

Fig. 5. Primary endothelioma of lymphnode. Compare this reticulum, which traverses the cell masses in all directions, with that of Fig. 6 and note its extensive branching and its gently curving course.

Fig. 6. Medullary type of adenocarcinoma of breast for comparison with Fig 5. This shows the greatest amount of invasion of the epithelial cell masses by reticulum that we have observed. There is a much straighter, simpler reticulum in this tumor.

Plate LXIX

Fig. 7. Malignant reticuloendothelioma. The reticulum is very intimately associated with the tumor cells in this case.

Fig. 8. Dural endothelioma. The coarsest, densest reticulum observed in any of our tumors; very few of these fibers are collagenous in nature.

Fig. 9. A fibroma molle of the vulva. The black fibers are reticulum, or partially impregnated collagen, the gray are collagenous.

Fig. 10. A soft, mucoid fibroma of external auditory meatus. No appreciable amounts of collagen, much fine reticulum.

Plate LXX

Fig. 11. A fibrosarcoma of the lung. The reticulum is fairly coarse and straight, and collagen is negligible.

Fig. 12. A fibrosarcoma of the knee. There is much collagen, which photographs grayish, and rather sparse reticulum.

Fig. 13. A fibrosarcoma of lung with marked anaplasia, but abundant collagen and comparatively little reticulum.

Fig. 14. A very rapidly growing fibrosarcoma of the scapular region, with extremely young and delicate reticulum and little collagen.

PLATE LXXI

Fig. 15. A liposarcoma of the sacral region. This shows very little, young reticulum. Note the cytoplasmic network. Little collagen.

Fig. 16. Lymphosarcoma originating in the thymus. It is typical of the lymphosarcomata we have examined from other localities. Very abundant reticulum, often young and beaded, and usually unassociated with large cells. Cf. Fig. 3.

Fig. 17. A section of lymphnode in Hodgkins' disease. This case shows little reticulum, probably all of it from the original lymphoid reticulum.

Fig. 18. A tumor from the parotid region, which is partly, if not entirely, epithelial in its make-up. It shows a very dense reticulum and well illustrates the relationship of epithelium to that tissue.

All these photomicrographs were taken at $\times 650$ diameters, 4 mm. objective and $\times 15$ ocular, with a short bellows (17 cm.).

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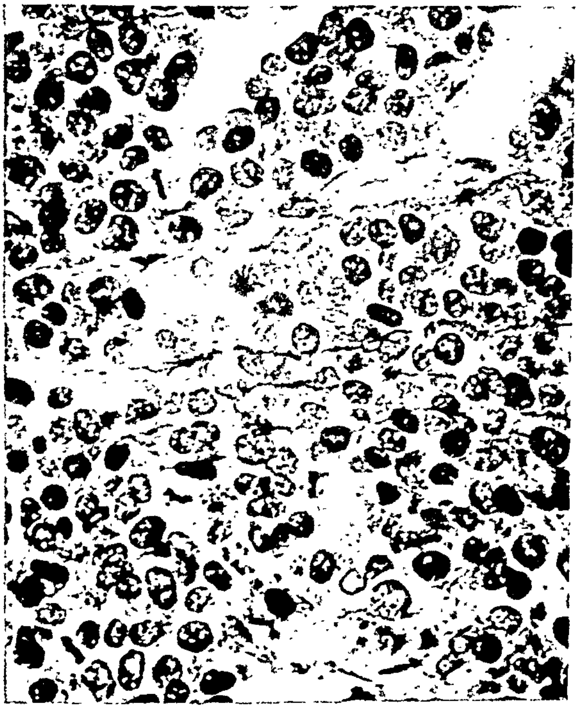

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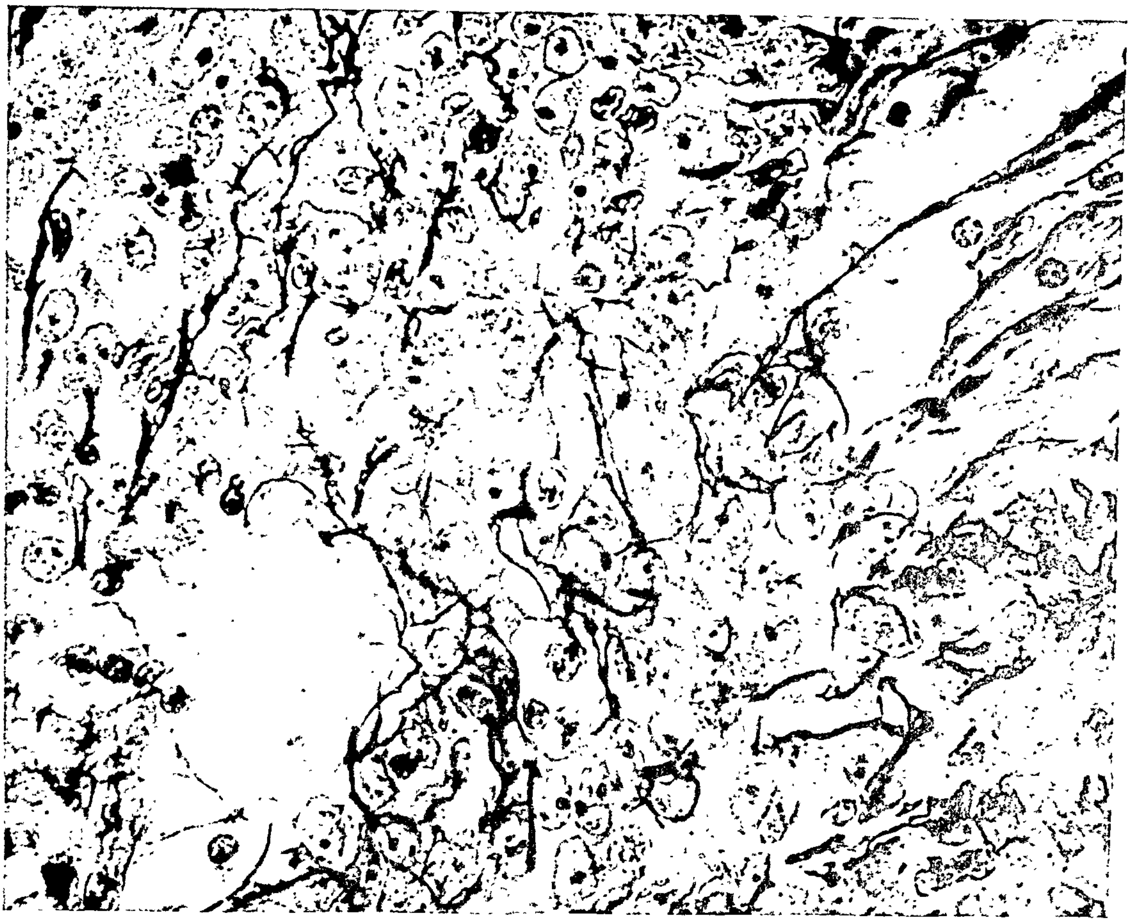

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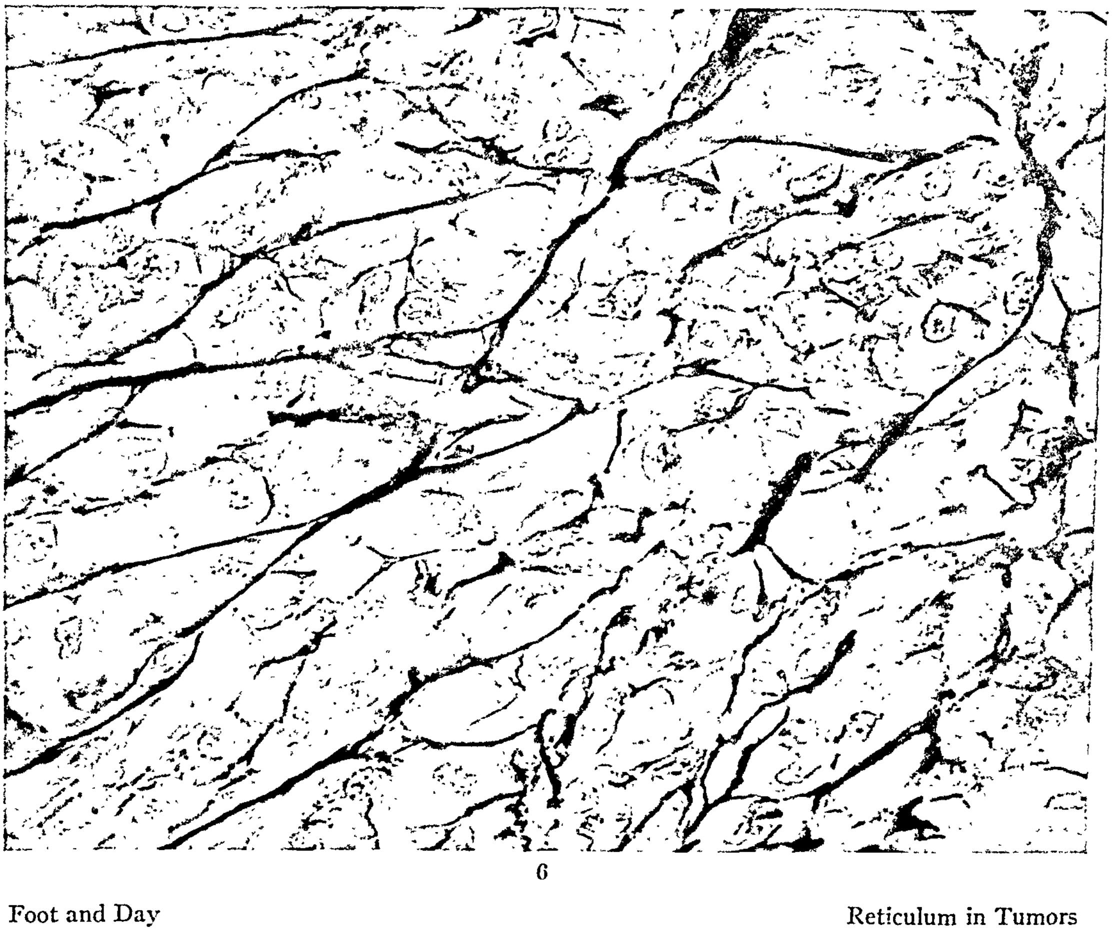

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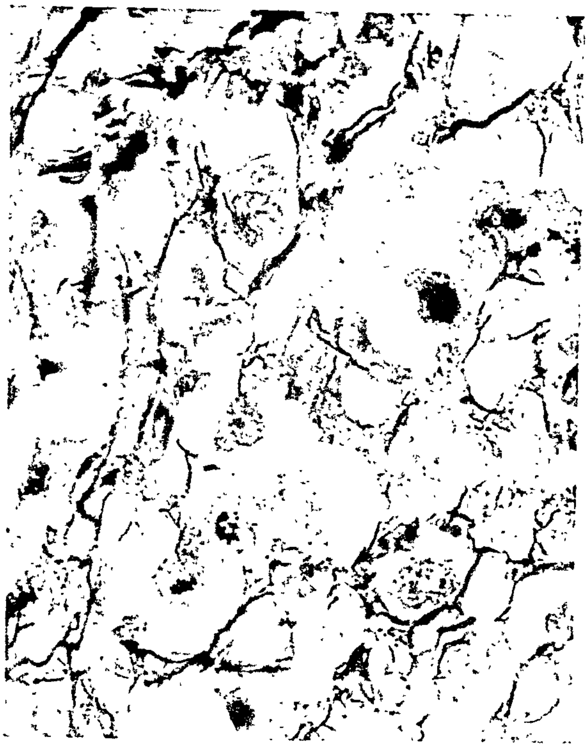

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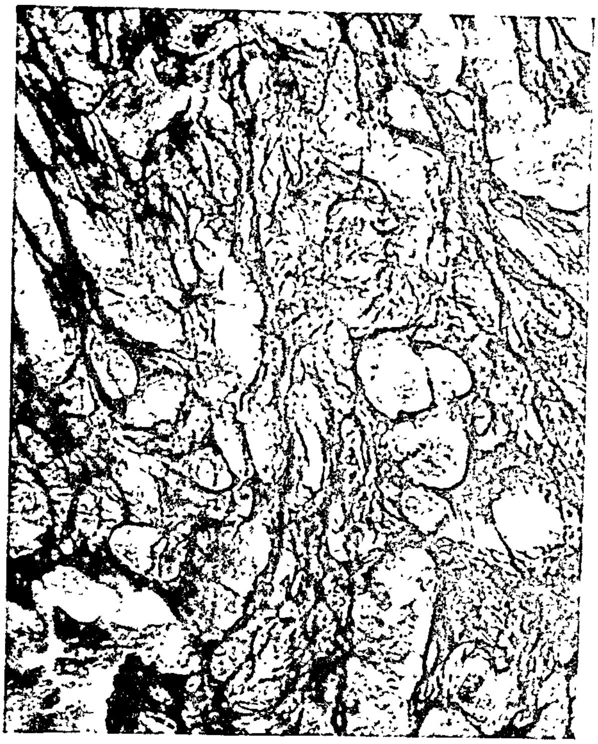

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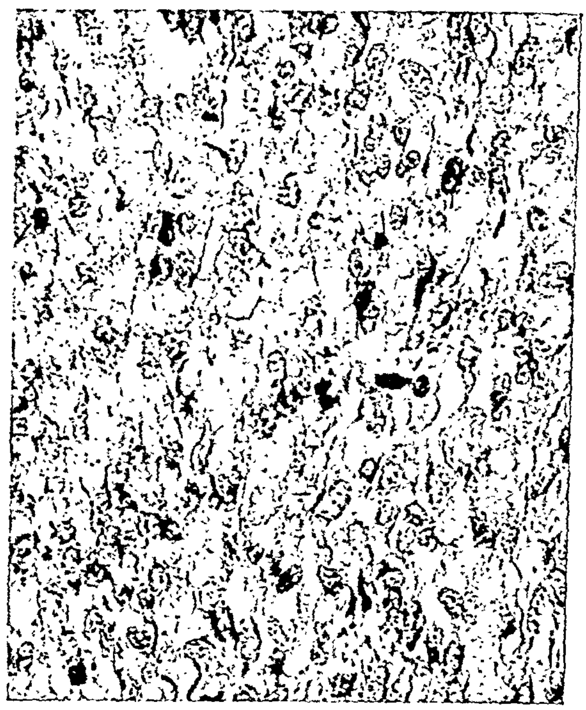

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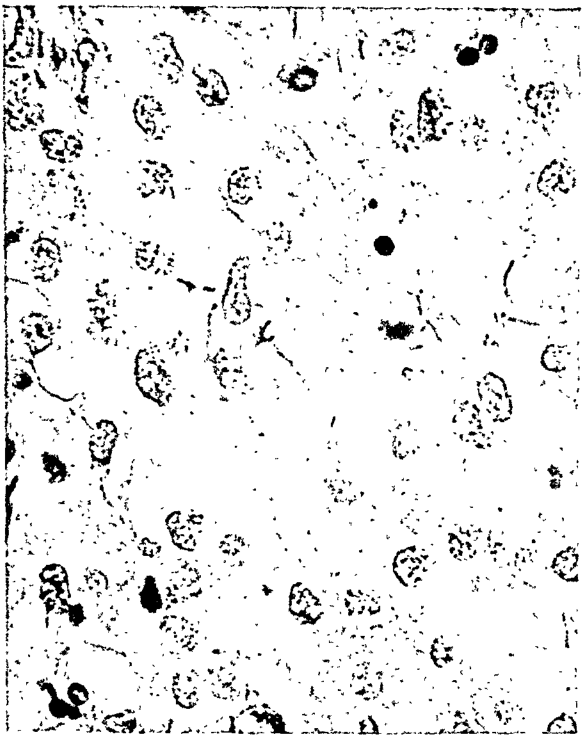
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INTRANUCLEAR INCLUSIONS IN VISCERAL DISEASE *

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The presence of intranuclear bodies with specific tinctorial properties has been widely accepted as indicative of infection with a virus having special affinity for tissues of neural or ectodermal origin. It is the purpose of this paper to present morphological evidence that similar bodies may be found in lesions of internal organs, and to bring forward for consideration the possibility that viruses of this type may localize in tissues other than skin and central nervous system.

Review of Literature

The history of such nuclear inclusions in mammalian tissue cells dates back to a publication by Jesionek and Kiolemenoglou [1] in 1904, on the finding of protozoön-like structures in the organs of an hereditary syphilitic. Their illustrations and descriptions show that they were observing bodies very similar to, or identical with, those with which this paper is concerned. They were found in the kidneys, lungs and liver of an eight-month syphilitic stillborn fetus. In the kidneys the cells containing them were irregularly scattered through the interstitial tissue of the cortex, often in groups of ten to forty. In the liver and lungs they occurred singly; in the lung they were found free in the bronchi and alveoli.

The intranuclear bodies are described as oval in form, with a fairly definite cuticular zone suggesting a capsule. The nucleus consisted of a central body, separated as if by a shell from the cytoplasm; on

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the inner surface of this shell (*i.e.* the nuclear membrane) were darkly stained granules of various sizes. The cell body was spongy and the pole near the nucleus filled with granules. As regards staining, the authors noted that the central body, that is to say the inclusion, stained rather palely and with a reddish tinge in hematoxylin-eosin preparations, after sublimate fixation, whereas the peripheral granules were dark blue.

The authors believed that they could exclude the possibility that these bodies arose as modifications from preformed structures. Their appearance suggested some animal or vegetable parasite, and after consultation with Professor R. Hertwig, it was concluded that they were probably Gregarines. The possibility that they were the cause of syphilis — this was before the days of the *Treponema pallidum* — was discussed, but not seriously entertained.

Shortly after the appearance of this paper Ribbert,[2] recalling that he had seen similar bodies some twenty years previously, published a short paper on protozoön-like cells in the kidney of a syphilitic newborn and in the parotid of children. In the kidneys Ribbert found large cells in the tubules but not in the glomeruli or stroma. The preparations were twenty years old and somewhat faded, but it was still evident that there were no transitions between these cells and the renal epithelium. They are described as having a round or oval nucleus containing a large homogeneous body, separated from the nuclear membrane by a clear zone. The cell body was slightly vacuolated or finely granular.

In Ribbert's second case similar intranuclear bodies were found in the parotid of a non-syphilitic year-old child. They occurred in the ducts singly or in groups, often pushing aside the epithelial cells. In his third case identical cells were found in even greater numbers in the parotid ducts.

As regards their significance Ribbert agreed with Jesionek and Kiolemenoglou that they were elements which in no way resembled any of the normal or pathologically altered body cells. This suggested at once their parasitic nature and the preparations were submitted to the zoölogists Rhumbler and Ehlers, who rather inclined to the opinion that they were amoebae or sporozoa.

In 1907, Loewenstein,[3] at Ribbert's suggestion, studied the parotids of thirty children from two months to two years old and found inclusions in four. In the last case they were observed in fresh un-

stained preparations. The slides were submitted to Professor Ludwig, the Director of the Zoölogical Institute, who was of the opinion that they were protozoa, either coccidia or other sporozoa. Loewenstein found no transitions to normal body cells.

Pisano,[4] in 1910, reported the finding of similar intranuclear bodies in the tissues of a stillborn fetus. The viscera in this case showed pronounced lesions — the liver, a gummatous hepatitis, while the kidneys, spleen, thyroid and lungs were all the seat of interstitial fibrosis. The large cellular elements containing inclusions were present in great number in the kidneys, fairly numerous in the liver and rare in the lungs. They were found in the crevices of the connective tissue and free in the lumina of the tubuli contorti of the kidneys. As to their significance various possibilities were considered and rejected. The most specious hypothesis, and the one adopted by Pisano, and also by Perrando [5] in a subsequent article, was that the cells were of epithelial origin but "arrested in their development by the dystropic and paraplastic effect of the syphilitic infection." The paper makes no reference to previous German observations. The following year Mouchet[6] found similar bodies in the bile ducts of an eight-day syphilitic infant, with icterus. They were regarded by him as sporozoa, the sporocysts of which were enclosed within hypertrophic epithelial cells.

Smith and Weidman [7] in 1910 published in the University of Pennsylvania Medical Bulletin a paper entitled "Infection of a Still-born Infant by an Amebiform Protozoan (Entameba mortinatalium), N. S." The bodies which they depicted and described were obviously identical in nature with those reported by Jesionek and Kiolemenoglou, and by Ribbert. One other fact of interest is brought out in their paper, that in the liver and kidney the supposed parasites were surrounded by a definite inflammatory reaction of lymphoid and polymorphonuclear leucocytes. In the light of subsequent events it is perhaps unnecessary to review the detailed steps by which Smith and Weidman excluded all the hitherto known amoebae as a result of which they were forced to create a new species for their parasite.

In 1914, the same authors,[8] having in the meantime become acquainted with the previous German reports, published a new observation. The case was that of a two-months' old child, dying of pneumonia. There were ulcerous eruptions about the mouth and nose and scaly lesions of the buttocks, no definite luetic parental

history, a negative Wassermann reaction, but fibrosis of thymus and of pancreas; so that there was a strong suspicion that the child, like the previous cases, was syphilitic. The "parasites," having the same appearance as those in the previous case, were found this time only in the lungs. There was an organizing pneumonia, and a few areas of caseous necrosis in which no tubercle bacilli could be found. The writers conclude that the "parasites" are "doubtless harmless for the mother, but for the fetus, especially when impaired by luetic taint, they may prove pathogenic and capable of destroying life."

Bodies of this type next reappear in the literature in 1921, being described this time as "An Intracellular Protozoan Parasite of the Duct of the Salivary Glands of the Guinea-pig." Leila Jackson [9] found it in 26 of 48 pigs examined, and described it as follows: "an encysted organism of irregular round or oval contour. In its most conspicuous and fully developed form, it practically replaces the host cell which still retains its relations to the duct wall. The center is occupied by a round or oval body, which stains deeply and unevenly, around which is a wide, lightly stained zone and outside of this a peripheral capsule." The illustrations which accompany the paper show a very close resemblance to the bodies described by the previous writers. Jackson, however, interpreted them as coccidial in nature, and thought they were situated within the cytoplasm of the epithelial cells, rather than within the nucleus. There may therefore perhaps be some question as to whether she was dealing with the same type of structure.

In the same year, 1921, Goodpasture and Talbot [10] published an excellent paper, "Concerning the Nature of the Protozoan-like Cells in Certain Lesions of Infancy." In a six-weeks' old male child, which had had green stools from birth, glucose in the urine, edema of the feet, and cough, they found large cells with acidophilic intranuclear bodies, in the alveoli of the lungs, in the chronically inflamed bronchi, and impacted in the glomeruli of the kidneys. Their illustrations leave no doubt that the bodies were identical with those in our own case, and with the bodies reported previously.

Goodpasture and Talbot remark that these inclusion-containing cells resemble no normal element of the body. They believe, however, that their origin can be traced to large mononuclear cells situated just outside the endothelium of small veins and capillaries, and that they may gain entrance into the blood stream, penetrating

the capillary walls. No cells of this type were observed in organs *other than the kidneys, lungs and liver.* The possibility that other types of cells, particularly epithelium, might be transformed into cells of this type, could not be excluded. Indeed, early stages of the transformation could be seen in the alveolar epithelial cells.

The authors found it difficult to form an opinion as to the nature of this remarkable change, which suggested the intranuclear bodies described by Tyzzer [11] (1906) in varicella. They felt very certain that whatever they might be, they were not protozoa.

De Lange [12] *in 1922* recorded a similar finding in a three-day old icteric infant with cirrhosis of the liver. Syphilitic infection could not be proved, the viscera showing no spirochaetae by the Levaditi method, and the parents giving a negative Wassermann reaction. The inclusion-containing cells were found in the convoluted tubules of the kidneys; in their vicinity, there was round cell infiltration of the stroma. They were interpreted as some undetermined form of cell degeneration.

Three further cases were reported in 1922 by J. Müller,[13] one in a stillbirth, one in a child eight weeks old with hydrocephalus and slight interstitial nephritis, and a third in a two-months' old child with congenital syphilis. The inclusions were found only in the kidneys; their appearance conforms to that described by previous observers.

Müller excludes the possibility that the cells are protozoan because of their occurrence in stillbirths, and because it does not seem possible that they could have passed the placental barrier. He is therefore forced to believe that they originate from tissue cells which have undergone a peculiar degeneration, characterized by the dissociation of the oxy- and basi-chromatin within the nucleus, and by hypertrophy of the affected cells as a whole. The foregoing cases are summarized in Table 1.

We should hardly be justified in reviewing these isolated findings at length were it not for the fact that the matter of these intranuclear bodies takes on new interest and importance with the discovery of B. Lipschütz [14] in 1921 that similar structures are constantly and characteristically associated with the lesions produced by the herpes virus, both in man and rabbits. Although it had been previously shown by Grüter [15] and Loewenstein [16], that the virus of herpes febrilis is transmissible in series to rabbits, Lipschütz was the

first to make a careful study of the intracellular inclusions in this disease, and in herpes zoster and herpes genitalis, and to interpret these bodies as the expression or result of an intranuclear virus. Since Lipschütz's first publication, numerous articles have appeared, dealing not only with the question of cell inclusions, but with the most interesting and still obscure problem of the relation of the herpetic virus, and the encephalitis produced by certain strains of it in rabbits, to the virus of epidemic encephalitis in man. We shall attempt to discuss only the herpetic inclusions, and their possible bearing on the interpretation of the case which is here reported.

Lipschütz showed first of all that these bodies are not artifacts, inasmuch as they may be easily recognized in fresh preparations. They are round, oval, or even slightly irregular structures ranging from 2 micra to such as completely fill the nucleus, reaching to the nuclear membrane but leaving usually a small clear zone. Smaller inclusions lie against a clear background. Usually there is only one within a nuclear membrane, but sometimes several. Practically every nucleus at the site of the herpetic lesions may contain an inclusion. Frequently the inclusions appear homogeneous, but in properly differentiated iron-hematoxylin preparations, they appear to be composed of numerous minute granules embedded in a homogeneous matrix.

Although they typically occur within the nuclei, they may in early lesions be occasionally found in the cytoplasm in the vicinity of the nuclear membrane. They are present both in the nuclei of epithelial cells, and in the swollen hydropic nuclei of connective tissue cells in the corium, and in the cells about blood vessels. Once an inclusion was seen within a mast cell, and once within the proliferating epithelium of a hair follicle.

The staining reactions are summarized as follows:

Stain	Inclusions	Nucleoli
Giemsa.	Red	Dark blue
Hematoxylin-eosin	Dark red	Blue-black
Heidenhain iron-hematoxylin	Yellow-gray	Black
Pappenheim	Green or blue	Red

The inclusions may thus be readily differentiated tinctorially from the nucleoli.

Lipschütz [14] discusses at length in this and a subsequent paper [17] the possible interpretation to be placed upon these and similar

TABLE 1

Résumé of Reported Cases

No.	Year	Author	Age	Pathological diagnosis	Location of inclusions	Interpretation
1	1904	Jesionek and Kiolemenoglou	Stillbirth	Congenital syphilis	Kidneys, lungs, and liver	Gregarines (R. Hertwig)
2	1904	Ribbert	Newborn	Congenital syphilis	Kidneys	Amoebae or Sporozoa? (Ehlers — Rhumbler)
3	1904	Ribbert	One year	—	Parotids	
4 5 6 7	1907	Loewenstein	Two months to two years	—	Parotids	Coccidia or other sporozoa (Ludwig)
8	1910	Pisano	Stillbirth	Congenital syphilis, Gummatous hepatitis, etc.	Kidneys, liver and lungs	Embryonic epithelial cells
9	1910	Mouchet	Eight days	Congenital syphilis	Bile ducts	Sporozoa
10	1910	Smith and Weidman	Stillbirth	Focal nephritis	Kidneys, liver, and lung	Entamoeba mortinatalium
11	1914	Smith and Weidman	Two months	Pneumonia	Lungs	Entamoeba mortinatalium
12	1921	Goodpasture and Talbot	Six weeks	Green stools, edema of feet, bronchitis	Lungs and kidneys	Degenerative change in the nuclei of endothelial leucocytes
13	1922	De Lange	Eight days	Congenital syphilis? icterus, cirrhosis	Kidneys	Undetermined form of cellular degeneration
14	1922	J. Müller	Eight weeks	Hydrocephalus, focal interstitial nephritis	Kidneys	Degenerative change, with dissociation of oxy- and basi-chromatin
15	1922	J. Müller	Stillbirth	?	Kidneys	
16	1922	J. Müller	Two months		Kidneys	

intranuclear inclusions, and reaches the conclusion that they represent the reaction of the nuclear plasm to a living virus classed with the Chlamydozoa-Strongyloplasma. To the group of these intranuclear viruses, which includes causative agents of the various forms of herpes, Borna's disease of horses (epidemic encephalomyelitis), varicella, fowl-pox, etc., is given the name "karyooikon group," distinguishing them from the cytooikon group, in which the inclusion is present in the cytoplasm. The trachoma bodies, the Guarneri corpuscle in smallpox, and the Negri bodies in the ganglion cells in rabies are representative of this latter group.

Lipschütz's arguments in support of the view that the inclusions are to be interpreted as a specific reaction to a living parasitic virus, are briefly the following:

1. Their general resemblance to the well-known inclusions of variola and vaccinia.
2. Their constant occurrence in the pathologic tissue, and their limitation to that tissue.
3. The impossibility of identifying them with hypertropic nucleoli or any known degenerative products of the cell.
4. The possibility of reproducing similar bodies by experimental inoculation of the virus.

Subsequent investigation has almost unanimously confirmed Lipschütz's work so far as the regular occurrence of the intranuclear bodies in spontaneous and experimental herpes lesions is concerned. There have, however, been differences of interpretation. Thus Lauda,[18] who studied the inclusions both in the herpetic lesion and within the ganglion cells and glia cells of the encephalitic rabbits, believes that the inclusions are the result of a degenerative, destructive process of the nucleus, whereby the oxychromatin comes to lie in the center of the cell, and the basi-chromatin moves to the periphery. Against the parasitic nature of the inclusions, he brings the following arguments: (1) the absence of evidence that the virus is really situated within the nucleus; (2) the absence of elementary bodies within the inclusions (he was unable to confirm Lipschütz's observation as to the presence of minute granules within the inclusion); (3) the lack of specificity, since bodies of apparently identical morphology are found in such diverse conditions as herpes labialis, zoster and varicella. Zdansky,[19] also, in a recent paper on

the pathologic anatomy of the encephalitis produced in rabbits by the herpes-encephalitis virus, ascribes no etiological importance to the inclusions found in the ganglion cells and glia cells, but regards them rather as degeneration products of the karyoplasma. Levaditi[20] and Da Fano[21] believe that the virus is more probably to be found in certain minute granular bodies scattered through the affected nerve tissue, both within the cells or between them. The larger intranuclear bodies (the "neurocorpuscules" of Levaditi, Harvier and Nicolau[22]) are regarded as products of nucleolar degeneration. Cowdry and Nicholson,[23] in studying cytologically experimental herpetic encephalitis material given them by Flexner, Noguchi and Amoss, attribute little importance to intranuclear inclusions of the Lipschütz type, and most of their attention appears to have been directed to the smaller types of granules emphasized by Da Fano. In conclusion, they state their belief that the inclusions which are so abundant in herpes do not represent a concrete class of granulations *sui generis*, but that they are of variable composition and derived from several sources.

Quite the opposite point of view is taken by Goodpasture and Teague. In one of the most recent of the brilliant series of studies in this field, Goodpasture[24] makes a very strong argument in favor of the view that the nuclear inclusions represent a change brought about specifically by the virus of herpes or closely related viruses. As the virus passes from the peripheral nerve endings to the central nervous system along the nerve trunks, a progressive involvement of the neurolemma cells and then of the ganglion cells in the corresponding region of the brain, may be demonstrated. When the lesion is unilateral, only the cells on the affected side show inclusions. While it is not maintained by Goodpasture that the intranuclear inclusion is itself the active agent of the disease, he does believe with Lipschütz that it indicates an intranuclear localization of the infective substance.

Case Report

F. S. (history 60991 — autopsy 9582), male, white, age 36 years. Entered Presbyterian Hospital September 27, 1924.

Chief Complaints. Fever for 3½ weeks. Pain in chest for 10 days.

Past History. Gonorrhoea 9 years ago. In 1908, an acute illness (appendicitis ?) since which time he has been subject to attacks of indigestion, characterized by acute pain across the upper abdomen and lower chest, coming on ½ hour after meals, and relieved by rhubarb and soda. At times the pain has been

localized in the right lower costal region and is relieved by lying on the affected side. No history of herpes was obtained on careful inquiry.

Present Illness. The present illness began gradually about one month ago with afternoon fever. Seven days after the onset a motile gram-negative bacillus was obtained from blood culture. This was at first believed to be *B. paratyphosus* but was later identified as *B. coli.* Ten days ago he began to have pains in the lower anterior part of the chest. The pain was burning in character and worst when fever was highest. There were no abdominal pains and no blood in the stools.

Physical Examination. Thin, well-developed man, acutely ill. Heart and lungs negative. Abdomen flat, with slight fullness in the right upper quadrant where there was definitely increased resistance and tenderness on pressure. Liver palpable 3 cm. below the costal margin. No abnormal neurological signs. Pulse regular, not dicrotic, 100. Blood pressure 105/55. Blood: R. B. C. 4,000,-000, Hg. 92 per cent, W. B. C. 16,900, P. M. N. 79 per cent, Lym. 20 per cent, Eosin. 1 per cent. Wassermann reaction negative. Stool negative for blood (Guaiac test). Blood culture: negative on one occasion; on another, *B. coli* was again recovered.

Course. The temperature curve suggested a pyogenic infection rather than paratyphoid fever. The tenderness in the region of the liver persisted, and the diagnosis lay between subphrenic and hepatic abscess. On October 9th, exploratory coeliotomy disclosed an abscess in the right lobe of the liver, which was drained. Culture of the pus yielded *Staphylococcus pyogenes albus.*

Operation was followed by only temporary improvement. Transfusion (750 c.c. of blood) failed to influence the temperature or leucocytosis. Examination of the chest was negative.

On November 4th, because of the failure to improve, he was again operated upon, and a subphrenic abscess found and drained. The septic temperature persisted. An X-ray on November 8th showed a shadow in the right lower chest and obliteration of the phrenic angle. Dullness was found over this area. The leucocyte count was 24,850, P. M. N. 86 per cent.

Upon aspiration of the chest, a small amount of blood and fluid, sterile on culture, was obtained. On a second aspiration of the chest 10 days later, a small amount of blood-streaked pus was withdrawn, from which was grown a non-hemolytic streptococcus in pure culture. The margins of the draining operative wound at this time had become necrotic over a wide area, and masses of necrotic liver tissue were discharged from the abscess.

On November 19th, a piece of tissue was removed from the wall of the liver abscess. Sections of this showed bile ducts, some compressed, some distended with polymorphonuclears. There was much dense granulation tissue in the midst of which were a few degenerating liver cells; also many phagocytes, some containing hemosiderin. No amoebae could be found. No organisms could be found in a Levaditi preparation. Smears from the abscess stained with methylene blue, and by Fontana method, showed many cocci, often in chains, and many bacilli but no spirochaetae or spirillae, or filamentous organisms.

On December 1st, the patient developed bloody diarrhea and on December 5th *amoeba histolytica* was reported as present in the stools. The administration of emetin hydrochloride (0.63 gm. in twelve days) was followed by a decrease in the number of the stools. No amoebae were found after the emetin therapy was begun. The margins of the liver wound showed signs of more active

healing, with the appearance of granulation tissue, and the abscess cavity became smaller. The fever, however, persisted, and also the pulmonary signs.

On December 18th he had two bloody stools, the second one consisting of almost 500 c.c. of blood. He became weaker, and died two days later, having been ill approximately 3½ months.

Autopsy 9582. Anatomic diagnoses: Abscess of the liver; ulcerative colitis with hemorrhage; suppurative pleurisy, right; lobular pneumonia, organizing; small bronchiectases; fibrous pleural and peritoneal adhesions; sclerosis of pulmonary venules.

The body is that of a fairly well-developed, moderately emaciated white man, 165 cm. in length. There are no cutaneous lesions. There is rigor of the jaw, neck and extremities, and some lividity of the dependent portions. There are no abnormalities over the calvarium; no discharge from the eyes, ears, nose or mouth. The pupils are round, equal and in mid-dilatation. The conjunctivae and buccal mucous membranes are quite pale. The teeth are in good condition. There is no enlargement of the thyroid; the superficial lymph glands are not palpable. The chest is long and narrow. On the right side of the chest, in the posterior axillary line, over the 8th rib, is a recent surgical incision, through which a finger can be introduced directly into the pleural cavity. From this wound there flows a small amount of thick, milky-white, purulent material. There is a long incision over the lower anterior chest on the right, following the course of the ribs. The cartilages of the lower ribs project into the wound and are necrotic. There is considerable erosion of the epithelium around the margin of this wound, especially at the inner angle, where there is a granulating surface 2 cm. in breadth, extending back from the edge. The wound is widely gaping, and through it one can see directly into a cavity which extends into the liver. In the upper right quadrant of the abdomen are two gray, glistening scars which touch each other at their upper ends and form an angle of about 60°. The external genitalia are normal. There is no edema of the feet or ankles. The subcutaneous fat is practically absent.

Abdominal Cavity. There is no excess of fluid in the peritoneal cavity. There are fairly recent adhesions between the almost fat-free omentum and the anterior abdominal wall, and also in the region of the cecum. These adhesions become denser in the region of the liver and the anterior surface of the right lobe is bound to the anterior abdominal wall by tough adhesions which completely wall off the abscess from the peritoneal cavity. The liver edge extends 9 cm. below the xiphoid.

The *liver* weighs 1480 grams and measures 20 x 20 x 10 cm. After dissecting off the adhesions, there is seen in the right lobe on the anterior surface a cavity with an opening about 2 cm. in its greatest diameter. The surface of the right lobe of the liver is for the most part devoid of adhesions except in the region of the cavity; the left lobe is covered with a smooth glistening capsule. There is no accumulation of pus between the liver and diaphragm. On section it is seen that the cavity extends into the liver for a distance of 3 cm. The wall is composed of gray, glistening, dense fibrous tissue, superficially bile-stained and covered with a little exudate. Above the cavity, which is in the dome of the liver, there is an infarct-like area of atrophy and congestion, slightly sunken below the level of the surrounding parenchyma. This wedge-shaped area has its base toward the capsule and its apex is against a large branch of the hepatic vein. Contrasting with this, the lobules in other portions of the liver are considerably

larger; there is a very narrow red zone about the efferent vein, but about the portal vessels is a broad grayish-yellow zone. Surrounding the abscess cavity and extending for a variable distance toward the inferior border on the anterior surface is again seen an irregular zone, identical in appearance with the large one described. A communication between the larger bile ducts and the abscess cavity cannot be demonstrated, but there are seen in the wall of the cavity several oval openings from 1 to 2 mm. in diameter, which appear to be the openings of bile ducts. The portal vessels and bile ducts elsewhere appear normal. There are no other abscesses or infarcted areas found. There is no general increase in connective tissue.

The *gallbladder* is small; its wall is thin. It contains a small amount of pale viscid bile.

The *spleen* weighs 225 grams and measures 16 x 7 x 3 cm. The capsule is smooth and glistening. The organ is moderately soft. On section the pulp is reddish-gray in color; the Malpighian bodies are small but easily seen.

The *pancreas, adrenals, kidneys,* and *pelvic organs* are normal.

Gastro-intestinal tract. The stomach, duodenum, jejunum and ileum are normal.

In the cecum are found discreet ulcers of varying size with clean bases; only a few of these show slight undermining. These ulcers extend down to the inner layer of muscle and tend to encircle the gut; they measure 0.5 cm. to 4 cm. in greatest dimension. The wall about them is not indurated, and clinging to the margin of one of these ulcers are blood clots. In the ascending colon also are a few ulcers, to one of which a blood clot is adherent. They are similar in appearance to those in the cecum, but somewhat smaller. The bases of the ulcers are covered neither by exudate nor by definitely recognizable granulation tissue. There are fifteen of these ulcers. No other ulcers are found in the remainder of the large intestine.

The *lymph glands* in the mesentery are not enlarged; on section they appear normal.

Thoracic cavity. There are numerous adhesions between the lower lobe of the right lung and the parietal pleura. These adhesions tend to wall off the small cavity communicating with the incision in the lateral chest wall. A few adhesions are present between the apex of the upper lobe on the left and the parietal pleura. There is no excess of fluid in either pleural cavity.

The upper lobe of the *right lung* is everywhere air-containing and fluffy, as is also the middle lobe. The lower lobe is much collapsed, dark red and flabby; its pleura roughened by the fibrous adhesions and by a fibrino-purulent exudate. On section the cut surface of the upper and middle lobes is pale. There is no consolidation. The lower lobe on section is dark red, firm, but not uniformly consolidated. The pleura is quite obviously thickened; it seems also somewhat wrinkled, yet the exudate fills in the spaces between these wrinkles, producing a level surface. Scattered throughout the lower lobe are small grayish areas which appear to be more or less confined to the immediate region of the smaller bronchi. The bronchi are not thick walled.

The upper lobe of the *left lung* is everywhere air-containing. The lower lobe is heavy. The posterior portion is dark bluish-red in color, and this area is somewhat sunken below the level of the air-containing lung adjacent to it. The lower lobe feels flabby; it does not have the consistence of a firmly consolidated lung but is more or less elastic. On section there are found in the

upper lobe a few areas, lobular in size, which are consolidated, and the cut surface of these is yellowish in color. In the lower lobe are small areas of consolidation which are for the most part close to the smaller bronchi; they rarely measure more than 0.5 cm. in diameter, and are not confluent. The bronchi in the centers of these in some instances contain a little purulent fluid, but for the most part they are empty and their walls are not especially thickened. Some of the medium-sized bronchi are dilated; they are, however, lined with smooth mucosa. The lymph glands at the hilum of the lung are not enlarged; they contain a considerable amount of pigment.

The *heart* and *aorta* show no significant changes.

Diaphragm. There is no demonstrable communication between the abscess in the liver and the right pleural cavity.

Bacteriologic report. The culture from the content of the large intestine was negative for dysentery bacilli.

Microscopic examination: Liver. The wall of the abscess is composed of dense connective tissue in its deeper part where there are many widely distended capillaries. At the outer margin of the abscess wall are small accumulations of liver cells undergoing atrophy, and also many mononuclear wandering cells of considerable size, many containing yellow pigment, others having vacuolated cytoplasm. Bile ducts are also found; some of these have a wide lumen, in others the lumen is very small. In the more superficial part of the abscess the wall is less dense; it is infiltrated with small round cells and a few polymorphonuclear leucocytes. Upon the surface are large colonies of cocci. Throughout the entire abscess wall are many large cells, often with basophilic cytoplasm, and having oval or round, vesicular nuclei. In each of these nuclei is a very prominent deeply staining mass. These cells are to be seen both within the capillaries and in the connective tissue. Frequently they appear to be passing through the wall of the capillary or are lying against the endothelial lining of the vessel; in places also they seem to have just penetrated the capillary wall and are lying immediately adjacent to it on the outer side. Some of the endothelial cells lining the capillaries are swollen. The liver lobules immediately about the abscess are flattened, and the liver cells atrophic. There is some increase in connective tissue in the portal areas near the abscess, and groups of phagocytes containing hemosiderin are to be seen. At a distance from the abscess the liver parenchyma appears normal. An occasional small accumulation of polymorphonuclear leucocytes grouped around a necrotic cell is found in the sinusoids; these degenerating cells are apparently not in continuity with the liver cells or endothelium of the sinusoids but lie free within the lumen.

Intestine. The ulcers extend down to the submucosa. The base is composed of loose granulation tissue infiltrated with small mononuclear wandering cells. In the granulation tissue and within small blood vessels are large cells with vesicular nuclei, in each of which is to be seen a very striking intranuclear mass. The infiltration with wandering cells does not involve the underlying muscle. In one or two instances an arteriole lying in the superficial part of the ulcer has undergone necrosis and the hemorrhage probably came from such vessels. There is no regeneration of the epithelium at the margin of the ulcer.

Lung. The smaller bronchi are filled with an acute inflammatory exudate and the epithelium has disappeared in many instances from at least a portion of the wall. In other places the epithelium has become flattened and more squamous in type. The walls of these bronchi are infiltrated with wandering

cells of all varieties, and the infiltration extends into the adjacent alveolar septa which are wide. Many of these alveoli are filled with polymorphonuclear leucocytes. In some of the bronchi the exudate has undergone organization and the lumen is partly filled with a fibrous plug. The exudate in some of the alveoli also is undergoing organization. The capillaries are engorged.

In other areas the alveoli are filled with a recent acute inflammatory exudate. The septa of these alveoli are not infiltrated with wandering cells. In many alveoli the epithelium is distinctly cuboidal, and apparently attached to or continuous with the lining epithelial cells are very large cells with vesicular nuclei, each containing a prominent, deeply staining mass. Some of these cells lie free in the alveoli or in the exudate within them. Large cells, at times multinucleated, with intranuclear masses, are found within the smaller branches of the pulmonary artery, or lying just beneath the endothelium of these vessels. These cells are identical in appearance with the striking cells found in the liver and in the intestinal ulcers.

In the section from the right lower lobe the pleura is greatly increased in width, and here is found granulation tissue which is very dense. The capillaries in the granulation tissue are quite large; adjacent to the capillaries, and at times within them, are to be found many of the large cells with the prominent intranuclear masses. The alveoli in the immediate vicinity of this granulation tissue have thickened walls, they are lined with cuboidal epithelium, and their lumina are much smaller than normal.

In the section from the left lower lobe, the pleura is only slightly thickened, and there is a delicate fibrinous exudate upon it. In many parts of the section the alveoli are filled with polymorphonuclear leucocytes, and the epithelial cells lining these alveoli are larger than normal, but less cuboidal than in many parts of the section from the right lower lobe. These epithelial cells are deeply basophilic in their staining reactions. The alveolar septa are frequently thickened. In many places the alveolar space is filled with edema fluid, and in such alveoli the lining epithelium is quite prominent. These alveolar septa are not especially thickened. In yet other alveoli is compact fibrin with a few polymorphonuclear leucocytes. The smaller bronchi are frequently dilated, and their lumina are filled with polymorphonuclear leucocytes; the epithelium has undergone metaplasia. Throughout the section are many of the prominent cells with the large intranuclear bodies. These are found in the smaller branches of the pulmonary artery, in the capillaries or just beneath the endothelium of these vessels, and also free within the alveoli or in direct continuity with the epithelial cells lining the alveoli.

With the Gram stain, many gram-positive diplococci, often in pairs, are seen in the areas of acute inflammatory exudate.

Spleen. In the pulp are many polymorphonuclear leucocytes and hemorrhages. None of the large cells are found.

Adrenals. The cortical cells are depleted of lipoid. In one of the capillaries is a single large cell apparently undergoing degeneration.

Testes. There is no spermatogenesis. The membrana propia of some of the tubules is thickened and the interstitium is edematous. Small accumulations of lymphocytes, many of which are undergoing karyorrhexis, are found in the interstitial tissue.

Heart, kidneys, pancreas, and *prostate* are essentially normal.

Nuclear Inclusions

In the foregoing protocol, brief reference has been made to the intranuclear bodies present in intestine, liver and lungs. They were found, as has been noted, in large cells for the most part, isolated from the surrounding tissue, although in the lungs they appeared in some cases to be continuous with contiguous alveolar epithelial cells. Even with low magnification the inclusion-containing cells were conspicuous by virtue of their large size, measuring 25 micra. Usually they contained a single nucleus, but cells with two, three or four nuclei were seen. In these multinucleated cells inclusions were sometimes present in only one or more of the nuclei (Fig. 3).

The inclusions themselves varied in size, shape and, to a certain degree, in their staining reaction. The largest forms measured 11 micra in greatest dimension and completely filled the nuclear area with the exception of a narrow, clear zone sharply separating them from the deeply stained nuclear membrane. Projecting into this clear zone from the nuclear membrane were the deeply stained remnants of the chromatin material, often aggregated into a single lenticular clump. In addition smaller and less definitely stainable chromatin granules could be seen lining the nuclear membrane on its inner surface. The smaller forms, of which there were often several within the nucleus, were at times difficult to distinguish from the nucleoli and, as will be pointed out later, stained less distinctively.

The shape was most commonly spherical or ovoid, but elongated sausage-shaped forms were seen (Figs. 1 and 2). The structures appeared to have a certain plasticity, conforming to the shape of the nucleus. They generally were sharply outlined, but occasionally their margin was somewhat fuzzy and indistinct. Forms which were interpreted as degenerative showed fusion with the nuclear membrane, the latter also becoming indistinct.

The bodies showed little evidence of internal structure. At times the central portion stained more intensely; at times also one could see irregular areas of rarefaction. By none of the staining methods used, including Heidenhain's iron-hematoxylin, was it possible to identify minute granules within the inclusions.

The staining reactions conform closely to those given by Lipschütz [14] in his original article on herpetic inclusions. In addition to the reactions given by Lipschütz, it was found that the bodies stained

brilliantly red with Mallory's acid fuchsin — aniline blue, orange G, and that they did not retain the gentian violet in the Gram stain. With the Levaditi stain they became a uniform chestnut brown in contrast to the yellow of the remaining tissue. With Bensley's modification of Altman's mitochondrial stain the inclusions were vividly red. The cytoplasm of the inclusion-containing cells stained purplish with the hematoxylin-eosin, being in general more basophilic than the surrounding tissue cells. The cytoplasm appeared finely granular; but indistinct and well-defined granules were demonstrated only with the Mallory stain, and when present they were intensely fuchsinophilic. These cytoplasmic granules were not found in all of the inclusion-bearing cells. Stained with Scharlach R, some of the larger cells in the lung sections were found to contain finely divided fat globules within the cytoplasm; these did not extend completely to the surface but left a clear superficial zone. The outline of the cell was usually sharp, but it was sometimes possible to discern a paler staining fringe suggesting an ectosarc. One or two cells were found with plasmatic pseudopods, devoid of granules.

Location and Origin of the Cells

In the intestinal lesions the inclusion-containing cells are found in the stroma of the granulation tissue forming the base of the ulcers. Frequently they are near the blood vessels and indeed are often seen lying immediately beneath the endothelium, penetrating it, or within the lumen.

In the liver, they are most numerous in the granulation tissue lining the wall of the abscess, both intra- and extra-vascularly. A few degenerating forms were found in the hepatic sinuses at a distance from the lesions.

In the lung they are found projecting from the alveolar wall and interposed between unaffected alveolar epithelial cells; free in the alveolar cavities; and in the walls of the arteries and veins and within and without the lumina of the alveolar capillaries (Fig. 4). They are also found in the granulation tissue of the pleura. The large forms are not present in the bronchial epithelium or within the lumina of the bronchi.

The bodies as they have been described above, present little difficulty in their recognition. A closer study of the sections, especially those of the lung, discloses the presence of smaller intranuclear

inclusions within a large proportion of the alveolar and bronchial epithelial cells. These are distinguishable with difficulty from nucleoli. In sections stained with aniline fuchsin-methyl green, however, they retain the fuchsin whereas the nucleoli of the bronchial and alveolar epithelial cells, studied in twenty other cases, failed to give this reaction. One is therefore led to believe that these smaller forms may represent early stages in the formation of the larger inclusions.

As regards the origin of the cells it seems certain that there is more than one type of cell affected. In the lung the large inclusion-containing elements are frequently seen in direct continuity with neighboring alveolar epithelium. On the other hand the presence of cells with intranuclear bodies in the granulation tissue and within the blood vessels points to another source, obviously not epithelial. So alien in their appearance, however, are these cells that it is impossible to assign to them a definite origin from any one particular type of mesenchymal element. There is a striking tendency of those cells which are not of epithelial origin to localize in the vicinity of the blood vessels, or immediately beneath the endothelium, or even within the lumen. This suggests, but does not establish, their origin from the adventitial wandering cells.

Discussion

Since there is no possibility of carrying on an experimental study with the material from this case, an interpretation of the nature and significance of the inclusions must for the present rest upon the basis of similar observations recorded by others. There can be no doubt that the inclusions are identical in their morphology and staining reactions with the bodies seen by previous observers in the viscera of infants, and by Lipschütz and others in the tissues of spontaneous and experimental herpes, and in the various neural and visceral lesions produced by the herpetic and related viruses. Various possible interpretations thus present themselves:

1. The bodies (including those occurring in herpes and related conditions) represent merely a peculiar form of nuclear degeneration, not produced by a specific virus.
2. The bodies indicate the localization of the herpes virus or a similar virus within the nucleus of certain visceral cells.

It seems hardly necessary to consider seriously the possibility that the inclusions themselves or the inclusion-containing cells represent protozoan parasites. The mere fact that bodies of this appearance can be produced at will in a variety of tissues by the injection of herpetic virus, effectually disposes of this theory.

While it may be admitted that no positive proof has yet been brought that inclusions of this type are due to an intranuclear virus, there are several strong arguments against the first possibility, namely, that they are merely non-specific nuclear degenerations. They are present in cells which are evidently actively mobile. It seems unlikely that cells showing such marked nuclear degeneration should preserve their capacity for ameboid motion. Furthermore, individual cells are selectively involved whereas in the ordinary degeneration large numbers of the cellular elements are simultaneously affected. Again there are no obvious degenerative alterations in the cytoplasm of the inclusion-containing cells apart from the occasional presence of fat. On the basis of the above considerations, and above all because of the infrequency of their occurrence in routine pathological material, it seems safe to infer that the inclusions are not produced by a banal, non-specific nuclear degeneration.

There is but one argument, a very convincing one, however, in favor of the second possibility, that the inclusions in this case are caused by a virus identical with or closely related to the herpetic group. That argument is the morphological and tinctorial identity of the structures with those occurring in spontaneous and experimental herpetic lesions. We have recently had opportunity to compare the inclusions with those produced in the rabbit cornea and brain by the inoculation of the contents of a vesicle from a case of herpes labialis, occurring in one of the laboratory workers. The inclusions correspond in every particular.

Recent work has made it probable that viruses of this nature are more widely distributed, and perhaps more persistent, than had hitherto been suspected. Although it could not be established that the virus isolated by Rivers and Tillett [25] from cases of varicella was actually the cause of that disease, the virus obtained did produce in the skin, testicles and corneae of rabbits intranuclear inclusion-bodies apparently identical with those described in herpes, and situated for the most part within endothelial leucocytes as well as within the epithelial cells. Most interesting also is the transmissible virus

obtained by Miller, Andrewes and Swift [26] from the testes of rabbits inoculated with the blood and joint fluids of patients suffering from acute rheumatic fever, which had the power of producing inflammatory lesions with typical nuclear inclusions in the testes, skin, pericardium and heart muscle of inoculated animals. Still more interesting and puzzling is the isolation by Andrewes and Miller [27] of an apparently identical virus from the testes of supposedly normal rabbits. Flexner and Amoss [28] have also reported obtaining a very virulent neurotropic virus from the spinal fluid of a case of vascular and neural syphilis.

That man may harbor the herpes virus in a latent state is suggested by the experiments of Bastai and Busacca.[29] They examined the blood and spinal fluid of a large series of patients who had had no herpetic eruption for a long period. Positive results were obtained by corneal inoculation in rabbits in a large proportion of cases.

We are obviously at the beginning of our knowledge of this interesting group of diseases and it would be ill-advised and premature to say that the case presented illustrates a hitherto unrecognized disease caused by a virus akin to the herpes viruses. And yet, after studying the rather confused literature in this field, it seems less improbable than it did at first glance. There is first of all the closest possible morphological identity between the nuclear inclusions found in our case and those which are coming to be generally recognized as characteristic of the herpes-encephalitis type of virus. Secondly, there is growing evidence that the lesions produced by viruses of this type are not necessarily restricted to neuro-ectodermal structures, as maintained by Levaditi,[30] but may under suitable conditions be produced in such diverse tissues as trachea, bronchi, ovary, testis, adrenal (Goodpasture and Teague [31]). The virus may be present in the blood, spinal fluid or buccal secretions, and may be introduced into the body by direct inoculation of liver, pancreas, ovaries, thyroid, salivary glands, kidneys and spleen (Teissier, Gastinel and Reilly [32]). Even if we assume, as is probable, that the peculiar large cells with inclusions are to be interpreted as an infection with a virus of this group, it is still impossible to prove that this virus is the primary cause of the intestinal ulcers, liver abscess and pneumonia. The presence of bacteria in the liver abscess and pulmonary lesions makes it still more difficult to arrive at a conclusion. In favor of the view that the supposed virus is concerned in the lesions are:

First, the occurrence of the inclusion cells in numbers only at the site of the lesions; second, the fact that viruses of this type are capable of inciting an inflammatory reaction, in some cases of considerable severity; and third, that we are unable to attribute the changes to any other discoverable agent.

We are indebted to Dr. Walter W. Palmer and Dr. Allen O. Whipple for permission to transcribe the clinical record, and to Mr. Alfred Feinberg for the drawings.

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DESCRIPTION OF PLATES LXXII–LXXIII

Fig. 1. Cell with intranuclear inclusion, continuous with lining epithelium of lung alveolus. (Hematoxylin-eosin stain.) X 1050.

Fig. 2. Inclusion-containing cell lying within capillary in wall of liver abscess. (Hematoxylin-eosin stain.) X 1050.

Fig. 3. Lung: Multinucleated cell in alveolus. Two of nuclei contain typical inclusions. (Eosin-methylene blue stain.) Oc. 10x. Imm. 1/12.

Fig. 4. Lung: Two inclusion-containing cells lying just outside endothelium of a capillary. (Eosin-methylene blue stain.) Oc. 10x. Imm. 1/12.

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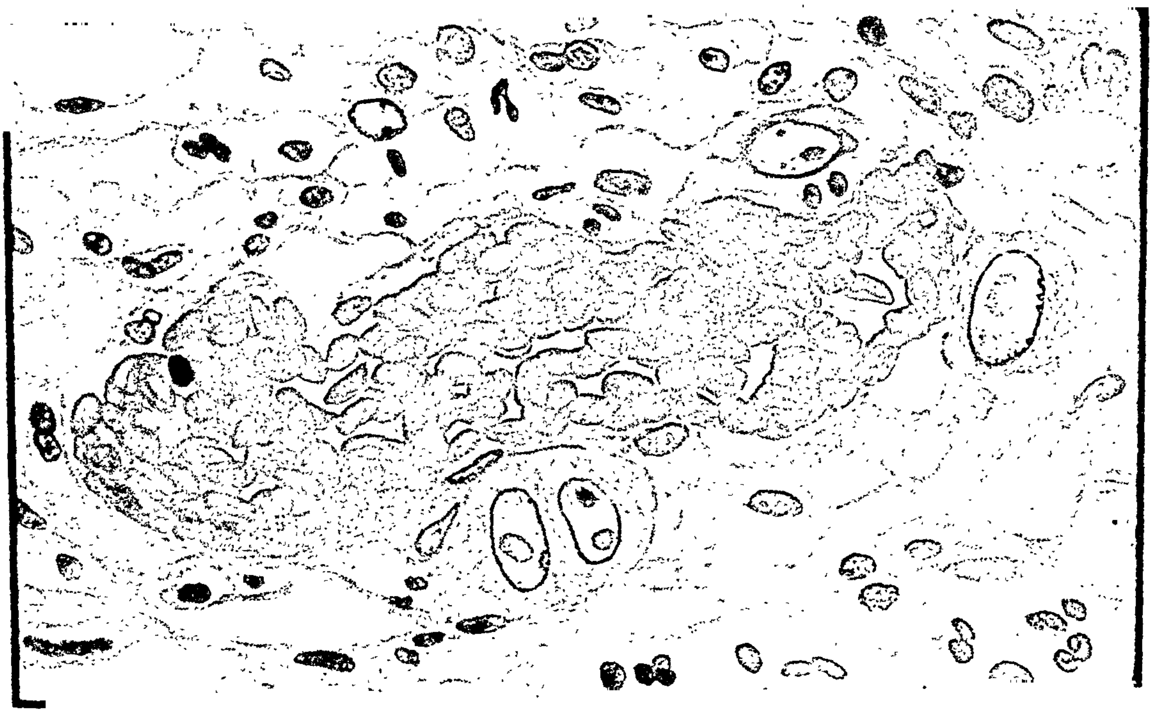

4

CONGENITAL HEART DISEASE WITH PARTIAL SITUS INVERSUS, ABSENCE OF INFERIOR VENA CAVA, AND OTHER ANOMALIES *

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Because of the unusual features of the case and the fact that a study of anomalies may help solve embryological phenomena, this report and discussion are submitted.

Patient, B.D.E., entered the hospital because of refusal to nurse, and cyanosis on crying.

Past History. The babe was full term and delivery spontaneous.

Birth weight, 7 lbs. Obstetrician noted no abnormalities. Breast fed.

Present Illness. Patient seemed well until after the first few weeks of life, when cyanosis was noted on crying. The attacks increased in frequency and degree. The patient gradually became more irritable and refused food. At the age of ten weeks rapid respirations were noted and the patient entered the hospital.

Physical Examination. Patient is a well developed, fairly well nourished baby girl ten weeks old. Cyanosis is marked in fingers and lips, slight on cheeks and forehead. Dyspnœic. Respiratory rate, 66 per minute. Cardiac dullness 1 cm. to the left of nipple line and 2.5 cm. to right of right sternal margin. Transverse cardiac dullness 8 cm. Signs of consolidation on lower right side of chest. Liver palpable on left side instead of right. Both heart sounds are altered and a murmur continues throughout cardiac cycle. Temperature 96°.

Clinical Course. Child refused to nurse, continued to be very irritable, and the cyanosis on exertion increased. Dyspnœa increased. Temperature rose to 96.6° on the day following admission and death occurred the second night.

Clinical Diagnosis. Bronchopneumonia, congenital heart disease.

NECROPSY REPORT

External Examination. Body is well developed and fairly well nourished, 54 cm. in length. There is a definite livid hue to entire body and less settling of blood posteriorly than usual. No edema. No discharge from any of the body cavities. Chest is rounded and full in upper part.

Peritoneal Cavity. About 200 c.c. (estimated) of clear, straw colored fluid. Right diaphragmatic dome is at the level of the 7th

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rib, and the left at the 7th interspace. Relations are as shown in Figs. 1 and 2, and will be described in detail below.

Chest Cavities. Pleural cavities are moist but there is no excess fluid. Parietal pleura is normal. Pericardial sac appears normal. It contains about 6 c.c. (estimated) of clear, straw colored fluid.

Mediastinum. Thymic tissue is recognized and appears normal in amount and consistence. Lymph nodes are slightly large and red. Relations described below.

The viscera are preserved *en bloc* for further dissection and study, and, therefore, not weighed.

Heart and Great Vessels. The position of the heart is normal. The great vessels are anomalous, as is shown in Figs. 3, 4 and 5. The

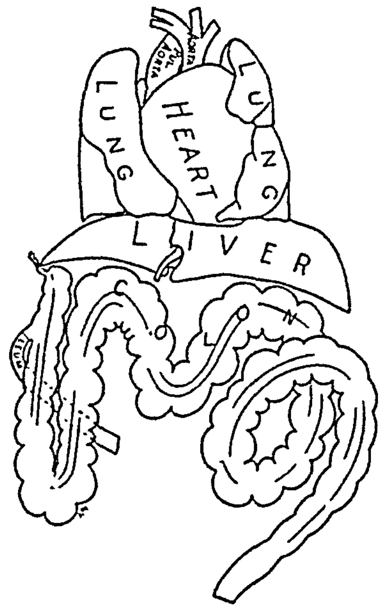

Fig. 1. Sketch at autopsy showing the redundant colon; the ileum passing posterior to the colon to enter it from the lateral side; the cecum and appendix in the right hypochondrium and the large left lobe of the liver.

common iliac and renal veins pass posterior to the aorta and empty into the azygos vein, which is normally placed but comparable to the inferior vena cava in size (Fig. 3). The inferior vena cava is absent save for the short vessel passing from the liver to the right atrium.

The systemic and pulmonic aortae are transposed and both arise from the left ventricle (Fig. 4). The pulmonary vessel is about twice the size of the systemic but the walls are about equal in thick-

ness. The right subclavian and right and left common carotid arteries are branches of a common stem. The left innominate vein does not join the right but passes downward, joins the coronary sinus, which is large, and empties into the right atrium (Figs. 4, 5 and 7). The ductus arteriosus (Fig. 5) is still functioning and measures 6 mm. in diameter.

The right atrium is dilated and hypertrophied, its wall being 1 mm. to 4 mm. in thickness. It receives blood from the superior and inferior venae cavae and the coronary sinus. Blood may leave this chamber through the patent foramen ovale, which is 13 mm. in diameter, through a large opening into the left ventricle and through a slit-like opening 1 x 3 mm. into the right ventricle (Figs. 6, 7 and 8). The atrial septum is not applied to the base of the ventricles but has a free margin. The annulus ovalis is absent in the anterior superior portion of the septum.

The right ventricle is small and under-developed, the outer wall being 2 mm. to 4 mm. in thickness. There are no papillary muscles in the right ventricle and its valve is incorporated with the mitral. The only entrance is the above mentioned slit. The only outlet is through the space where the pars membranacea septi failed to form.

The left ventricle is dilated and hypertrophied, its walls being 8 mm. to 12 mm. in thickness, and the moderator bands are very large. The mitral and tricuspid valves are fused into a large funnel-shaped curtain attached at the base of the ventricles and emptying into the left ventricle (Figs. 7 and 8). There are three partially formed leaflets, a right and left anterior and a posterior one. A row of papillary muscles is attached by long chordae tendineae to the left leaflet and a part of the posterior one. The right leaflet has its base attached to the outer wall of the right ventricle, covers the right chamber completely save for the slit-like opening, lies across the free margin of the ventricular septum and is attached on the left side of the ventricular septum by short chordae tendineae and miniature papillary muscles. The left ventricle has two outlets, the systemic and pulmonic aortae, the latter being to the right of the former (Fig. 9).

One coronary artery arises from the left posterior sinus of the aorta. The right and left coronary arteries are branches of this one vessel and the left gives off the circumflex branch. The final distribution is similar to the normal (Fig. 5).

The direction of muscle fibres is similar to the normal.

Lungs. The lungs are atypically lobed and fissured. They are dark, mottled red in color, firm, not crepitant save for a small part of the right upper lobe and the anterior part of the left upper lobe.

Liver. The liver, gallbladder, stomach, duodenum, pancreas and spleen are transposed so as to form an optical image of the normal, as is shown in Fig. 2. The left lobe of the liver is large; the hilum, gallbladder and spigelian lobe are to the left of the umbilical cleft. The inferior vena cava extends from the right lobe of the liver to the right atrium. The common duct empties into the second portion of the duodenum anteriorly.

Pancreas. The head is in the arch of the duodenum and the tail extends to the left. The ducts of the ventral and dorsal portions both persist, the latter emptying caudad to the ampulla of Vater instead of cephalad, as usual. The ventral duct joins the bile duct at its ampulla.

Spleen. There are six separate masses of splenic tissue (Fig. 3). Four are small and spherical, measuring 2 mm., 2 mm., 4 mm., and 10 mm. respectively in diameter. The remaining two are similar to the normal in shape, measuring 3.5 cm. x 2 cm. x 1 cm. and 4.5 cm. x 2.5 cm. x 1 cm. respectively. The hila face a common center. The largest one has a deep transverse fissure.

Gastro-intestinal tract. The esophagus is slightly to the right of the trachea, instead of to the left, which is in keeping with the transposed position of the stomach (Figs. 2, 3 and 4). The duodenum arches to the left so that the second portion is to the left of the second lumbar vertebra. The common duct enters the second portion anteriorly and is joined at the ampulla by the duct of the ventral pancreas (Wirsung's duct). The accessory pancreatic duct (duct of Santorini), or the dorsal pancreatic duct, enters medially and caudad to the ampulla of Vater, forming an ampulla in the mucosa.

The small intestine lies coiled in the lower and right part of the abdomen. The root of the mesentery extends downward and to the left, but is almost horizontally placed.

The colon is redundant. The cecum and appendix are in the right hypochondrium, extend upward and are in relation to the right small lobe of the liver. From here the colon passes downward, then left and upward, forming a "U" loop. The ileum passes upward, under both limbs of the "U" loop in the first portion of the colon, to enter

it laterally, as shown in Fig. 1. The transverse colon is long and sags. The portion of the colon in the left hypochondrium forms a complete spiral turn and then passes downward in the usual way.

The kidneys, ureters and pelvic viscera are not remarkable.

Pathologic diagnoses. Bronchopneumonia, bilateral, confluent; malformations of heart, great vessels and colon; situs inversus of liver, gallbladder, stomach, duodenum, esophagus, pancreas and spleen; persistent dorsal pancreatic duct.

Discussion

Normally, the inferior vena cava is a composite vessel, being formed from below upward by the union of the following vessels: the lower right veins of the prevertebral plexus (formerly this portion was thought to be a part of the posterior cardinal); the lower part of the right subcardinal; vein of the plica vena cava (caval mesentery) and the primitive right vitelline. The kidney develops opposite the anastamosis of the subcardinals in tissue containing a venous plexus which drains into them. A second venous plexus is found posterior to the above mentioned one and is called the prevertebral plexus. If the kidney develops around the latter plexus instead of the former it is possible for its vein to drain into the posterior vessels which form the azygos [1] and it will then be the exact condition described above. The subcardinal as well as the posterior cardinal veins have atrophied. It is also possible that the kidney developed in its normal position and the atrophy of the subcardinals occurred first, forcing the kidney veins to drain into the prevertebral plexus.

The position of the aortae is such that the systemic vessel is entirely on the left side. This may suggest that the first portion of the arch was derived from the left ventral aorta and the fusion and atrophy of the ventral aortic septae and possibly the rotation failed to occur. It is, however, not possible to trace either of the ventral aortae to the adult heart. In order to bring the aortae in proper relation in the above case, there must be a dextral tortion of 270°. It is interesting in this connection to remember Rokitansky's [2] sixteen possible forms of transposition, some of which were found after he had described them. Another possible explanation of aortic transposition has its foundation in the possibility that the first and second portions of

the adult aorta are normally a composite vessel and that the twined structures do not depend entirely on tortion for their relations.

The common stem for the three arteries, right subclavian and both common carotids, is a very common anomaly in man, occurring once in ten according to Parsons,[3] and is normal for rodents and other animals. The normal condition for the llama and giraffe is similar, differing only in this, that the common carotids have a long bicarotid stem. Morever, it is possible, as suggested by Lewis,[4] that the arrangement is a sign of Nature's effort to maintain symmetry which is so nearly, but not entirely, attained in the sheep. The arrangement is properly ascribed to migration of the left common carotid. The reason for migration is obscure, but the fact of migration is clearly shown by Jackson.[5]

The course of the left innominate vein is plainly the persistence of an embryonic structure, the left anterior cardinal vein. The anastomosing branch between the anterior cardinals, which develops to carry the blood from the left into the right, was not found. Its failure to form or to develop, as the case may be, would seem to explain the persistence of the distal portion of the left anterior cardinal and the course of its blood through the left duct of Cuvier (left common cardinal vein continued). The absent anastomosis may be result and not cause.

The ductus arteriosus is also a persistent embryonic structure — the distal part of the left sixth aortic arch. Whether or not the narrowing of the aorta in the second portion of the arch proximal to the opening of the ductus is an effect or a cause of its persistence is not demonstrated. In case of atresia of either aorta, the function of the persistent duct might seem to be clear. But in case of constriction of the aorta distal to the duct, its persistence on the ground of furnishing necessary blood is not explained. Such a condition is recorded at Iowa City.[6] In a series of 142 cases of aortic constriction mentioned by Abbott,[7] the ductus arteriosus was patent in only thirteen.

The lack of development of the right ventricle seems to be the result of a lack of opportunity for work. The only inlet is small and insufficient to carry blood to the ventricle, while its only outlet, the unclosed pars membranacea septi, would be closed during systole by pressure of the valve leaflet on the free edge of the septum. The enormous left ventricle might be thought of as a result of overwork, since the right ventricle is practically cut out of the circulation and

the heart functions as a bi-atrial-triloculate heart. The large septal defects allowing free passage of blood from one atrium to the other would seem to equalize the work of them. In this connection it is interesting to note the cases cited by Abbott,[7] as recorded by Simmonds,[8] Effron,[9] Kalb [10] and Ratner,[11] in which there was cardiac congenital hypertrophy without apparent cause. Hypertrophy without demonstrable cause is also found in adults, but is frequently spoken of as "so-called idiopathic hypertrophy." Certainly all of the influences which bring about hypertrophy are not clear.

The position of the atrial septum is normal. The interatrial foramen (Foramen primum) resulted from a failure of fusion with the base of the ventricles. The septum is smooth on either side and may represent the septum primum with an arrest of development before the formation of the posterior portion and a failure of formation of the septum secundum. The foramen ovale is not yet completed, since the antero-superior part of the annulus is absent. The opening here, then, is the foramen secundum, and should be called "foramen ovale" when the annulus is complete.

The ventricular partition is incomplete in that the pars membranacea septi failed to form and the muscular portion is placed to the right of its usual position. If the ventricular septum had grown from the tubercle between the aortae (Fig. 9), the functioning, assuming the presence of atrio-ventricular valves, would have been perfect and the condition would conform to the type, "corrected transposition," as described by Rokitansky.[2]

The formation of one valve at the base of the ventricles was probably influenced by the malposition of the ventricular septum.

The transposition of a part of the viscera is interesting in that it indicates that the forces causing rotation are probably different for the different viscera. The dextral rotation is in keeping with the common direction of the spiral in gastropod mollusks, yet a few of these turn sinistrally. However, the forces at work in these lower forms are also obscure. It may be suggested that in the above case the position of the esophagus is secondary to that of the stomach. The stomach may depend on the direction of the arch of the duodenum, which in turn may be dependent on the development of the liver and gallbladder. The hilum, being to the left of the umbilical fissure, is in keeping with the notion that the persisting vessels on the left instead of on the right have influenced the left part of the

liver to develop larger. The efferent vessel (vena cava), however, leaves the right lobe as normally.

The accessory pancreatic duct is the persistence of the dorsal pancreatic duct, as described by Santorini. It departs from the normal in that it has developed caudad to the ventral pancreas instead of cephalad, as usual. The placement of the ampulla of Vater and the duct of Wirsung (ventral pancreatic duct) cannot be explained on a simple basis of sinistral rotation.

Summary

The case presented may be summarized as: congenital heart disease comprising a patent foramen primum and secundum, patent pars membranacea septi, one atrio-ventricular valve emptying into the left ventricle, misplaced ventricular septum, both aortae arising from the left ventricle, hypertrophy of the left ventricle and right atrium; anomalous vessels comprising absence of inferior vena cava with the renal and common iliac veins emptying into the azygos vein, absence of the transverse portion of the left innominate vein which empties into the coronary sinus, a common stem for the right subclavian and both common carotid arteries, transposition of the aortae, persistent ductus arteriosus, and both coronary arteries arising from a common stem; *situs inversus* of liver, gallbladder, hilum of liver, esophagus, stomach, duodenum, pancreas and spleen; accessory pancreatic duct and spleens.

Explanation is offered as involving the following processes:

1. Persistence of embryonic structures, namely, interventricular foramen, foramen primum (interatrial foramen), foramen secundum (later called the foramen ovale), left anterior cardinal vein, dorsal portion of left sixth aortic arch (ductus arteriosus), dorsal pancreatic duct (duct of Santorini), branches of prevertebral plexus forming the renal veins.

2. Failure of development, namely, anastomosis of the anterior cardinal veins forming the left innominate vein; the lower right veins of the prevertebral plexus, right subcardinal, vein of plica vena cava, and primitive right vitelline vein forming the lower part of the inferior vena cava; one of the atrio-ventricular valves; septum secundum; posterior part of septum primum; pars membranacea septi.

3. Misplacement, namely, left common carotid artery, ventricular septum, ileum, lower part of ascending colon, cecum and appendix.

4. Reversed rotation, namely, esophagus, stomach, duodenum, pancreas and spleen.

5. Development of accessory structures, namely, multiple spleens.

6. Hypertrophy of azygos vein, left ventricle and right atrium.

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DESCRIPTION OF PLATES LXXIV — LXXVII

Fig. 2. Drawing of the ventral view of the transposed abdominal viscera. They form an optical image of the normal. The left lobe of the liver is large. The gallbladder, hilum and spigelian lobe are to the left of the umbilical cleft. Both pancreatic ducts persist. Sp., spleens; St., stomach; Li., liver; S. L., spigelian lobe; P., pancreas; D.V.P., duct of ventral pancreas (Wirsung's); D.D.P., duct of dorsal pancreas (Santorini's); D., duodenum; P. V., portal vein.

Fig. 3. Posterior view of viscera of the trunk. The common iliac veins and renal veins empty into the azygos vein. The esophagus is slightly to the right. L.C.I.V., left common iliac vein; R.C.I.V., right common iliac vein; L.U., left ureter; R.U., right ureter; L.R.V., left renal vein; R.R.V., right renal vein; A., aorta; A.V., azygos vein; R.I.V., right innominate vein; E., esophagus; L.S.A., left subclavian artery; L.C.C.A., left common carotid artery; L.I.V., left innominate vein; D.A., ductus arteriosus.

Fig. 4. Ventral view of chest viscera with right lung retracted. The right atrium and left ventricle are hypertrophied and dilated, the right ventricle rudimentary. The aortae are transposed. R.A., right atrium; R.V., right ventricle; I.V.G., interventricular groove; L.V., left ventricle; L.I.V., left innominate vein; L.S.A., left subclavian artery; L.C.C.A., left common carotid artery; T., trachea; E., esophagus; R.C.C.A., right common cartid artery; R.S.A., right subclavian artery; R.I.V., right innominate vein (superior vena cava.)

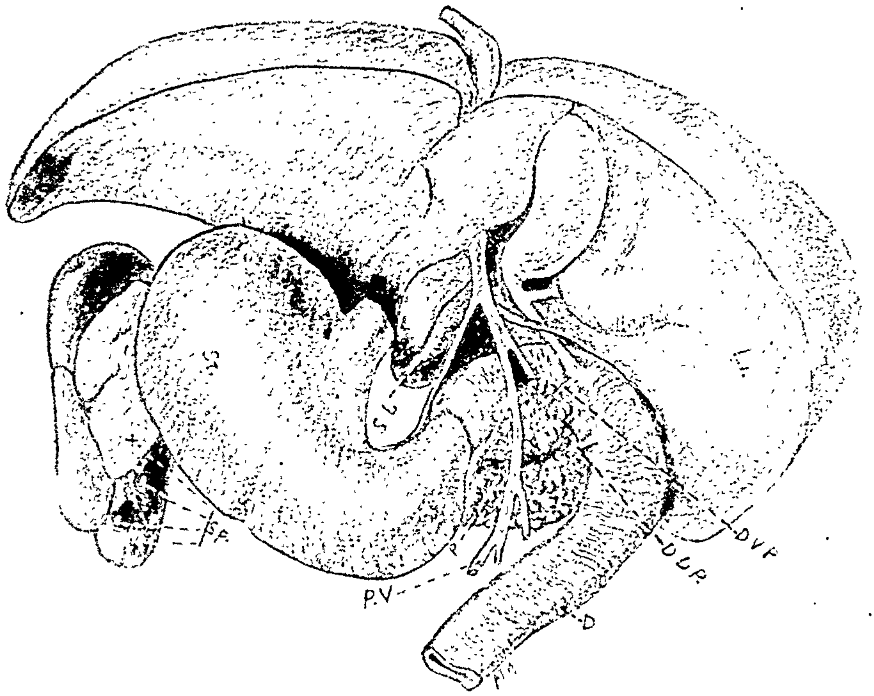
Fig. 5. Left view of heart and great vessels with a window in left atrium which is rudimentary. The two orifices in the atrial septum have persisted. The left innominate vein still joins the coronary sinus. The ductus arteriosus persists. Three large arteries arise from the aorta by a common stem. The coronary arteries arise from a common stem. C.S., coronary sinus; I.V., inferior vena cava; L.P.V., left pulmonary vein; P.A., pulmonary artery; D.A., ductus arteriosus; L.S.A., left subclavian artery; L.I.V., left innominate vein; A., aorta; L.C.C.A., left common carotid artery; R.C.C.A., right common carotid artery; R.S.A., right subclavian artery; F.O., foramen ovale; F.P., foramen primum; C.A., coronary artery.

Fig. 6. Right view of heart and great vessels with window in right atrium showing atrial septum. I.V., inferior vena cava; S.V., sinus venosus orifice; F.P., foramen primum; R.V., right ventricle; L.V., left ventricle; F. O., foramen ovale (foramen secundum); R.A., right auricle; R.C.C.A., right common carotid artery; R.S.A., right subclavian artery; L.C.C.A., left common carotid artery; R.I.V., right innominate vein (superior vena cava); A.V., azygos vein; P.A., pulmonary artery; P.V., pulmonary vein; P.S., indicates plane of section of heart for Figs. 7, 8 and 9.

Fig. 7. Anterior view of posterior portion of heart sectioned as shown in Fig. 6. The under developed and over developed portions of the heart are shown. The atrio-ventricular valve is funnel-shaped and empties into the left side. A slit-like opening allows some blood to go into the right ventricle, which has no outlet save through the patent pars membranacea. The chordae tendineae attachments are shown here and in Fig. 8. L.V., left ventricle; L.A., left atrium; C.S., coronary sinus; P.V., pulmonary vein; A.S., atrial septum; R.A., right atrium; I.V.C., inferior vena cava; S.V., sinus venosus; S., slit-like opening in atrio-ventricular valve opening into right ventricle; V. S., ventricular septum; R.V., right ventricle.

Fig. 8. Posterior view of anterior portion of heart. V.S., ventricular septum; R.V., right ventricle; A.V.V., atrio-ventricular valve; F.O., foramen ovale; A.S., atrial septum; A.L., auricular lumen; R.A., right auricle; R.I.V., right innominate vein (superior vena cava); P.V., pulmonary vein; P.A., pulmonary aorta; A., aorta; L. A., left auricle; C.S., coronary sinus.

Fig. 9. Same as Fig. 8 with the valve flap dissected off to show the orifices of the aortae and the free edge of the ventricular septum, which is not in the same plane as the atrial septum, being misplaced to the right. V.S., ventricular septum; A.V.V., atrio-ventricular valve; P.A., pulmonary aorta; A., aorta; O.P.A., orifice of pulmonary aorta; E.C., endocardial cushion; O.A., orifice of systemic aorta.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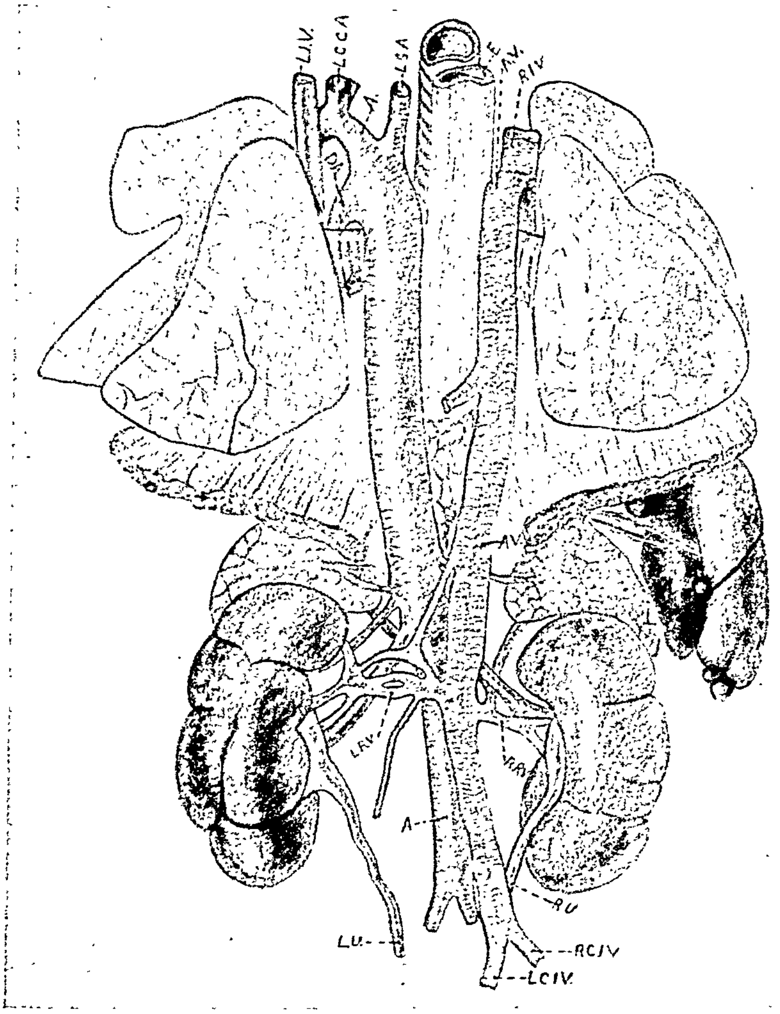
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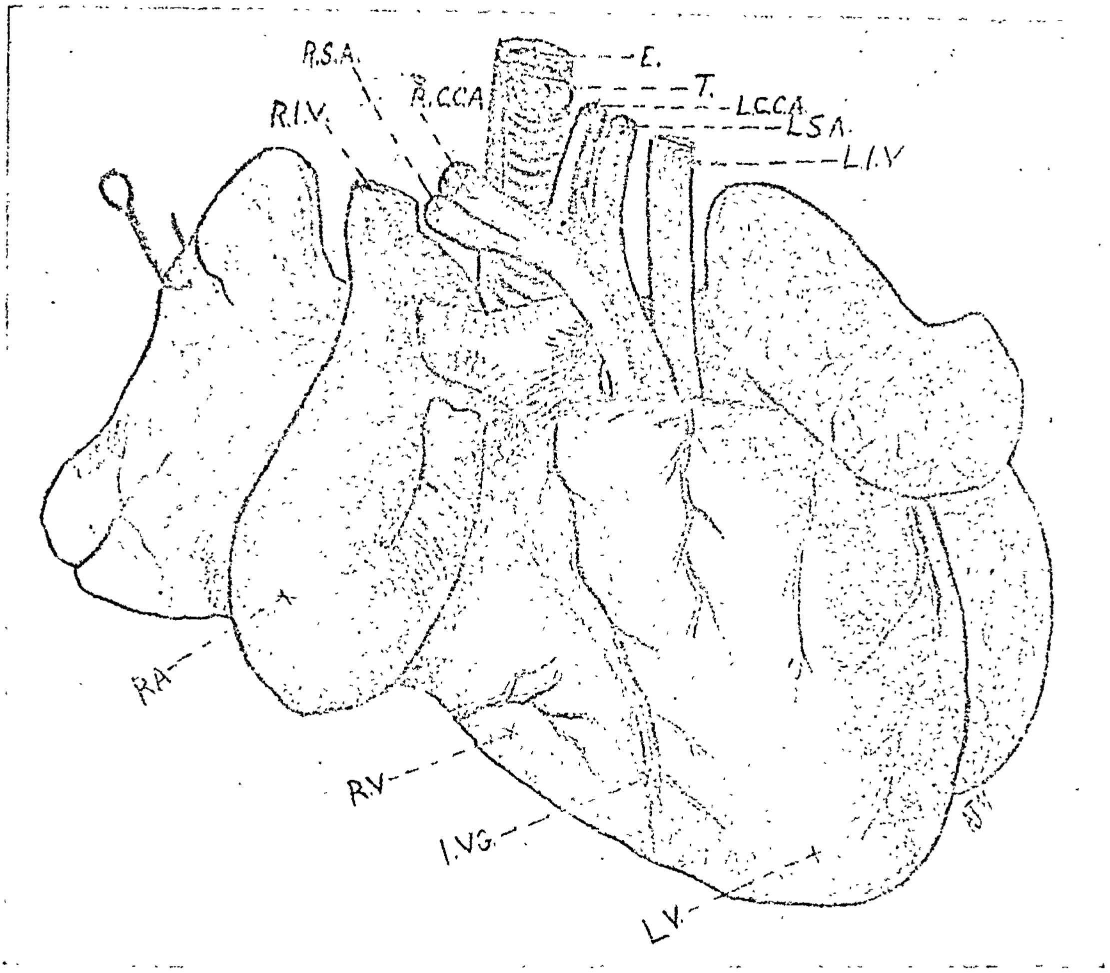
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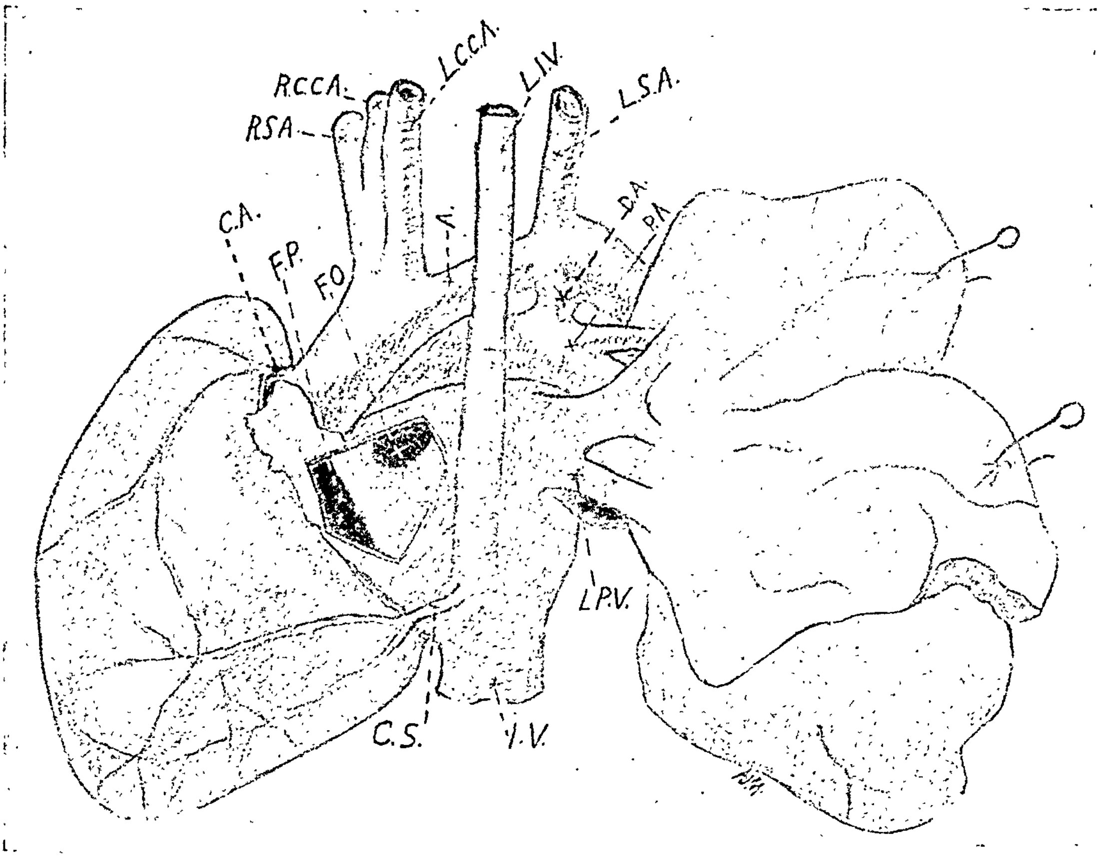
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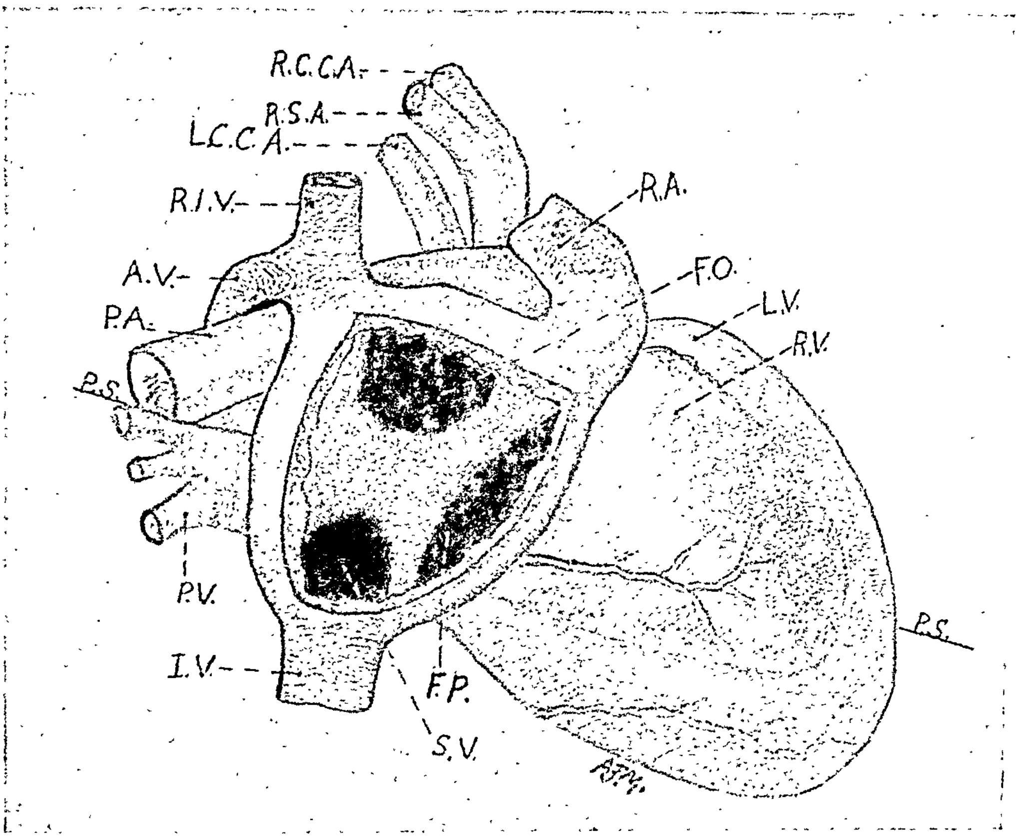
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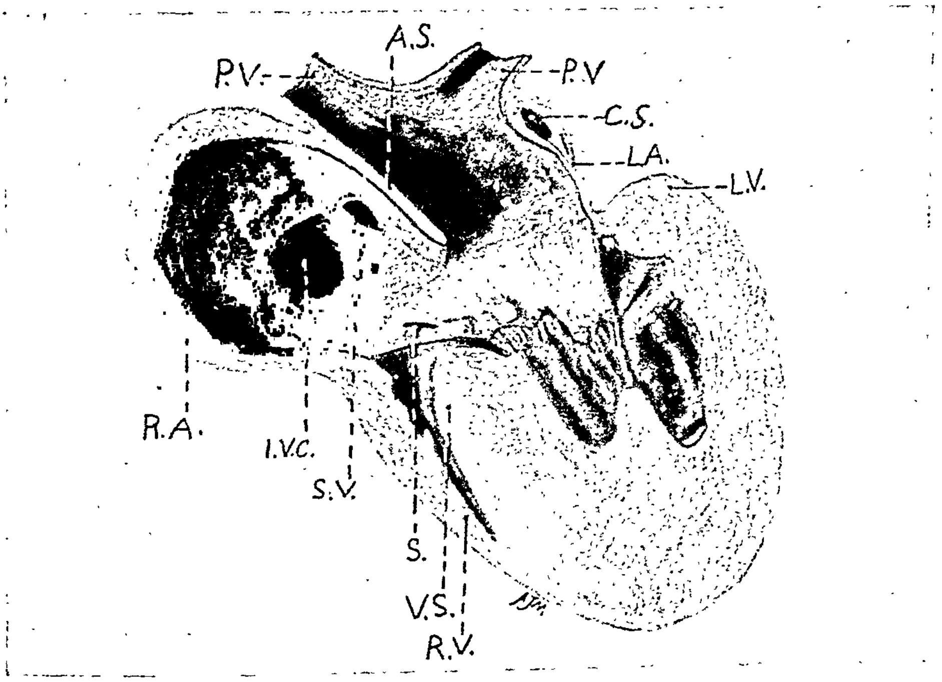
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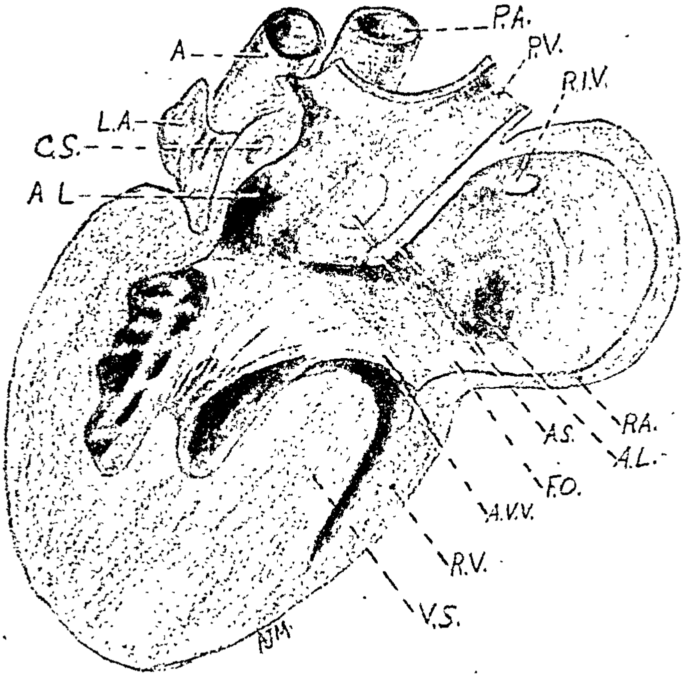
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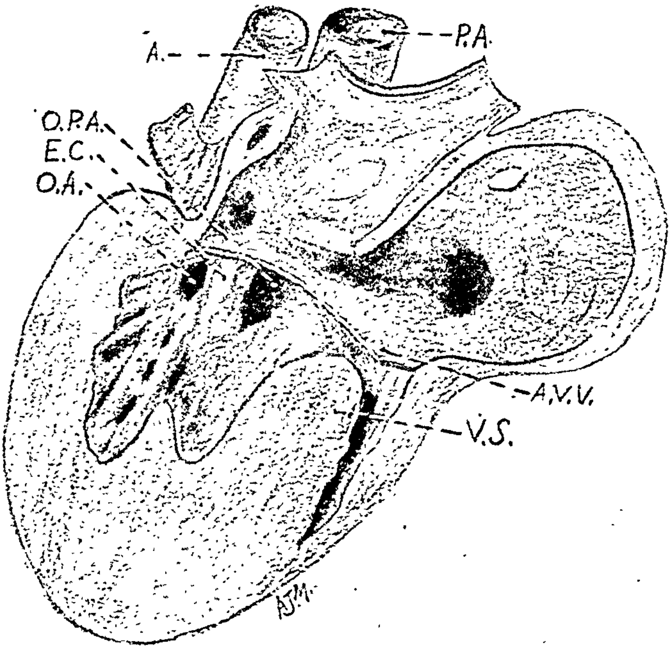
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9

PROGRESSIVE ALCOHOLIC CIRRHOSIS *

REPORT OF FOUR CASES

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(From the Department of Pathology of the Stanford University Medical School)

Since the time of Vesalius, who was the first to describe cirrhosis, the literature on the subject has been accumulating. Although volumes have been written, most accounts deal with the end stages of the disease. This seems the more remarkable when one considers that a study of the earlier stages gives greater promise of revealing the true nature of the process than does a study of the late stages. As Mallory [1] says, "In some organs the late stages of certain lesions have received more attention than the beginning of these lesions. The emphasis has been placed on the wrong end of the process. The statement is particularly true of the inflammatory changes in the liver and in the central nervous system." It is with these facts in mind that the present study is presented.

Diligent search of the literature has brought to light only a very few papers which deal specifically with the progressive stages of cirrhosis. Hawkins [2] regards the process as essentially inflammatory. He says, "If specimens of early cirrhosis are examined from cases in which death has occurred from other causes, no doubt can be entertained that the interstitial change is essentially an inflammatory one, and that it has its starting point around the branches of the portal vein at a time when the appearance of the degeneration of the hepatic tissue is either scanty or absent." Hawkins evidently underestimates the importance of the degenerative processes in the liver cells.

Mallory [1] in his study of the early lesions has paid particular attention to the peculiar form of necrosis of the liver cells which occurs in alcoholic cirrhosis and which seems to be characteristic of it. "The cytoplasm of the cell first undergoes a degenerative change in consequence of which an irregular, coarse, hyaline meshwork appears in it. . . . These processes (hyaline degeneration ending in necrosis, leucocytic infiltration, regeneration of liver cells, increase in the amount of connective tissue) when extensive, diffuse, and acute,

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lead to considerable increase in the size and weight of the liver." Mallory[3] states further, in his textbook, that there is a primary injury to the liver cells which is followed by more or less regeneration of them. In this he is in accord with those who have emphasized the importance of the regenerative processes in the disease. He goes on to say, "On the other hand, we have in alcoholic cirrhosis, around and invading each necrotic cell, an acute, inflammatory exudate of leucocytes which must cause more or less stretching of the connective tissue." This injury, he believes, results in the proliferation of fibroblasts which is mechanical, rather than toxic in origin.

Stengel and Kern[4] are doubtful as to the relative importance of the degenerative and inflammatory processes in the early lesions of cirrhosis. They say, "Fibrous tissue hyperplasia follows the injury to the parenchymal cells and may be simply a replacement fibrosis. In some cases, however, the evidences of inflammatory change (interlobular round cell infiltration, congestion and beginning fibrosis) are out of proportion to the evidences of cellular degeneration."

From these brief references it seems evident that there is still considerable difference of opinion in regard to the exact mode of development of the histologic changes in the liver in this disease.

Source of Material

In the present study four cases of cirrhosis showing relatively early progressive lesions in the liver have been utilized. These were selected from among about 150 cases of cirrhosis in over 3000 autopsies performed in the Department of Pathology of the Stanford University Medical School. The case histories and autopsy findings have been summarized and a careful histologic study of the liver made in each case.

Case 1

XX. 43. M.S., a white washerwoman, age 39 years, came to the San Francisco Hospital complaining of being unable to do sufficient work to support herself.

She had been married for eight years but later had separated from her husband. Two children had died of "spasms," one at one year, the other at 14 months. One child, 14 years old, is living. According to the patient's statement she had had no miscarriages. She denied ever having had any venereal infection.

She had never had any symptoms suggestive of disease of the liver. About one month before her entrance the patient began to cough and raise a small amount of sputum. She had lost weight since the onset of the cough, had felt weak and had tired very easily. Her voice had been husky for the past two

years but had not grown worse. The woman stated that she had used alcohol quite steadily for several years, wine with meals and one or two drinks of whiskey every day.

On clinical examination she proved to be a well-developed but undernourished woman of about 40 years. Her pupils were irregular and did not react to light or accommodation. Examination of the chest revealed the usual signs of pulmonary tuberculosis.

The abdomen was symmetrical. The liver dullness extended from the fifth rib to 12 cm. below the costal margin in the right mid-clavicular line. The lower border was notched and felt like liver edge; the surface was rough but not distinctly nodular. The liver was tender. The knee jerks were somewhat exaggerated.

The blood picture was normal except for a slight leucocytosis. The hemoglobin content was 95 per cent. The red blood cells numbered 4,500,000 per c.mm. and the white blood corpuscles 14,000 with a normal differential count. The urine contained a cloud of albumin and many pus cells. No red blood cells or casts were found in the sediment. Examination of the sputum revealed the presence of tubercle bacilli. The Wassermann reaction of the blood was triple positive.

Clinical diagnoses. Pulmonary tuberculosis, syphilis, cirrhosis of the liver.

Anatomic diagnoses. Syphilis of the aorta; aneurism of the arch perforated into the left bronchus; hemorrhage into the lungs; syphilis of the spinal cord; tuberculosis of the lungs, chronic; bronchiectasis; alcoholism, chronic; cirrhosis of the liver; carcinoma of the uterus (cervix); peritonitis, pelvic; suicide with a cutting instrument, attempted.

Abstract of autopsy record. The body is that of a strongly built, rather poorly nourished woman. The liver projects four fingerbreadths below the free margin of the ribs in the right mammillary line and two in the left. No fluid is present in the peritoneal cavity. The heart is less than one-half normal size; the heart muscle is thin and flabby. The wall of the aorta at its base is very much thickened, measuring about 3 mm. in thickness. The intima is full of small yellow spots and contains some scars.

The bronchi are filled with blood. Scarring and old caseous areas are found in the region of both apices with some recent tubercles in other parts of the lungs. The tonsils are small; the left one contains a small cavity filled with thick, viscid pus.

The arch of the aorta shows very marked thickening and extensive cicatrization. On the lower surface of the arch is an aneurismic sac about 7 cm. in diameter. The aneurism has perforated into the left bronchus.

The spleen is normal.

The cervix uteri shows a deep ulceration with indurated base.

The stomach contains some clotted blood. The gallbladder and biliary passages are normal.

The *liver* is rather large, measuring 26 x 26 x 7 cm. The surface is slightly granular. The liver tissue is quite firm, cutting with some difficulty. The cut surface shows a slight thickening of the periportal connective tissue.

Histologic examination. Microscopic examination of the cord reveals an early syphilitic inflammation of the meninges. Similarly sections of the aorta show the usual lesions of a syphilitic aortitis.

Liver. The capsule is of normal thickness. Within the liver there is a moderate increase in the periportal connective tissue. The new fibrous tissue has a patchy distribution and is arranged in roughly triangular or star-shaped areas from which finer fibers radiate out into the parenchyma enclosing small groups of liver cells (Fig. 1). The enmeshed liver cells show evidences of degeneration. Many of these cells contain hyaline masses in their cytoplasm; in others the cytoplasm is coarse, granular and vacuolate. The nuclei are irregular in shape and often swollen. They stain unevenly or in many cases they are absent altogether. Although the cell destruction is not so pronounced as in some of our other cases nearly every fibrous area contains groups of disintegrating cells. The development of collagen fibrils in the various areas of fibrosis is moderately advanced but the fibroblastic cells are still large and numerous.

There is evidence on every hand of widespread hyperplasia of the liver cells. Many areas are seen in which the cells have every mark of having been recently formed; they are well-filled, turgid cells; the cytoplasm is finely granular; the nuclei are of uniform size and staining. Many other cell-groups are found in which the cells appear to be recently formed but have a very pale cytoplasm which seems to be indefinitely vacuolate. These are, probably, cells loaded with glycogen. There is also a well-marked, rather diffuse, fatty infiltration of the liver cells as can be seen in Fig. 1.

Practically all of the new fibrous tissue is heavily infiltrated with polymorphonuclear leucocytes and lymphocytes. In many places the infiltration is quite marked and, as nearly as one can estimate, there are approximately equal numbers of neutrophilic polymorphonuclear leucocytes and lymphocytes.

Most interesting are the foci where the lesions are evidently quite recent (Fig. 2). Here many of the liver cells show hyaline degeneration and beginning necrosis. The spaces between them are being invaded by proliferating fibroblasts and there is a more or less heavy infiltration of the diseased tissue with polymorphonuclear leucocytes. The changes in the connective tissue are quite evidently of an inflammatory nature.

The "bile ducts" show only slight evidence of proliferation in or near the fibrous areas in this liver. Although there is evidence of widespread regeneration of the liver cells there is little to indicate that the "bile ducts" have played any appreciable rôle in the process.

The whole picture of this liver is one of considerable cell destruction and of extensive regeneration of the liver cells with a recent well-marked progressive inflammatory fibrosis. Some fatty infiltration is also present.

Bacteria were searched for in sections of liver stained with the Giemsa stain but none was found.

Case 2

XVII. 37. O. G. was an American teamster, 38 years old, single. He came to the San Francisco Hospital complaining of pains in the abdomen and swelling of the legs.

He had had "malaria" three years before and had been troubled with headaches for the past six months. His present illness began one month ago with pains in the abdomen and swelling of the legs. When the patient arrived at the hospital he was in a stuporous condition and his mentality remained clouded until his death. He had been a heavy drinker and he had been drinking to excess immediately prior to his entrance to the hospital.

Clinical examination showed jaundice. The liver edge was palpable and evidences of collateral circulation could be seen in the anterior abdominal wall. Ascites was marked and eight liters of clear fluid were removed by paracentesis. The calves of his legs were tender. The knee jerks and ankle jerks were absent on both sides.

The patient gradually grew worse and died as a result of his severe alcoholic intoxication.

His blood showed a slight secondary anemia and, toward the end, a high leucocytosis. The hemoglobin content was 80 per cent. The red cells numbered 4,000,000 in a c.mm. The Wassermann test of the blood and spinal fluid was negative.

Clinical diagnoses. Carcinoma of the liver; chronic alcoholism; alcoholic polyneuritis; Korsakow's psychosis; wrist and foot drop.

Anatomic diagnoses. Alcoholism; alcoholic psychosis; alcoholic neuritis; atrophy optic nerve; gastritis, chronic; cirrhosis of the liver (subacute); varices in the lower esophagus; pancreatitis with fat necroses; ulcers of the leg, healed; exophthalmus; tuberculosis of lungs, chronic, healed.

Abstract of autopsy record. The body is that of a fairly strongly built, extremely emaciated man. There is marked jaundice of the entire body. The left optic nerve shows a nearly complete gray atrophy. The abdomen is much distended laterally and contains about three liters of clear, greenish-yellow fluid with a few flakes of fibrin. The peritoneum is slightly hyperemic and moist. The liver extends slightly below its normal position. The heart is about three-quarters normal size.

There are old adhesions about the spleen which is slightly enlarged, measuring 12.5 x 10 x 4.5 cm. Its cut surface shows passive congestion and normal markings. There are some adhesions at the tail of the pancreas and the peritoneum over both kidneys is thickened. The lymph nodes at the hilum of the liver are slightly enlarged and bile-stained. The gallbladder contains thick, viscid, clear bile. The bile ducts are normal and patent.

The capsule of the *liver* is fairly normal except on the lower surface of the

left lobe where there is slight diffuse thickening with more marked fibrosis about the lymph vessels. The liver is somewhat above normal size, measuring 27 x 22 x 10 cm. The surface is smooth and of a greenish-gray color in which there are a large number of small, bright, yellow spots 1–2 mm. in diameter. The liver cuts with great difficulty. Its cut surface is smooth and shows numerous yellow spots with narrow gray bands between them. The liver substance is very heavy.

The pancreas is somewhat indurated and full of small fat necroses, the largest about 3 mm. in diameter.

Histologic examination.

Liver. The capsule is regular and of normal thickness, while the liver parenchyma presents a variegated picture. The lobules are greatly broken, in many places almost destroyed. Replacing the liver cells or ramifying among them are strands of recent, very cellular connective tissue with a marked cellular infiltration consisting largely of polymorphonuclear leucocytes (Fig. 4). This connective tissue is little beyond the fibroblast stage. The new connective tissue appears quite edematous. The periportal connective tissue is increased considerably in amount. It also is very cellular, yet it is somewhat older than the connective tissue penetrating the lobules proper. In lobules in which the central vein is intact it is surrounded by recent fibrous tissue which is connected with the periportal tissue by means of radiating strands. The amount of fibrous tissue varies greatly in the different lobules, but in all, the amount is relatively large. In many there is very little left but fibrous tissue, leucocytes and a few scattered liver cells. In others, perhaps one-third to one-half of the lobules are composed of hepatic cells grouped closely together (Fig. 3).

In the lobules, poor in liver cells, the cells are mostly pale and swollen. The cytoplasm is vacuolate, partly granular and partly hyaline. The nuclei are swollen and somewhat irregular in shape and staining. In some cells no nuclei can be found. In other words these cells are undergoing a peculiar form of degeneration ending in necrosis. In the larger cell-groups the liver cells are compact. Their cytoplasm is finely granular, staining well with eosin. Their nuclei are round, regular and take the nuclear stains well. A moderate number of these cells are binucleate and a few may be found with three nuclei. No definite mitotic figures are seen. The nucleolus is prominent and usually eccentrically placed. The chromatin appears to be somewhat more concentrated about the nuclear membrane and

about the nucleolus with a fine network connecting the two. The cells have the appearance of vigorous, regenerating cells.

In the periportal connective tissue are many proliferating "bile ducts." These appear as round or oval rings, or as long hollow tubes according to the way in which they happen to be cut. The epithelial cells lining them are packed closely together so that the nuclei appear to touch or actually to overlap one another. The cells are cuboidal in shape; their cytoplasm is basophilic, small in amount, and the cell boundaries are often indistinct. The nuclei are oval and stain deeply. No definite nucleolus can be made out. The chromatin is distributed as a fine network through the nucleus with somewhat thickened intersections.

The liver cells with their large amount of deeply staining cytoplasm, round nuclei, each with its nucleolus, are readily differentiated from the epithelial cells lining the "bile ducts," even though the two types of cells may be mixed quite indiscriminately. In many places small groups of liver cells which stain perfectly and which appear to be recently formed are scattered among the new "bile ducts" and are often arranged in the same form as the "ducts." Occasionally one sees a "bile duct" in which the cells seem to be undergoing transformation. At one end one sees typical bile duct epithelium, at the other end typical liver cells with cells in between which are not distinctly of either type.

Study of the circulatory apparatus shows large dilated veins in many of the periportal spaces with the compact groups of regenerating liver cells almost invariably located near these vessels. In many of the lobules the central vein cannot be found. In a number of places the bile capillaries are distended and filled with bile.

The whole picture, then, is one of a rather intense subacute inflammatory condition as shown by the edema, the large number of polymorphonuclear leucocytes and the widespread recent connective tissue proliferation. The simultaneous presence of marked destruction of the liver cells without evidence of pressure or other mechanical injury, suggests the direct action on them of some toxic substance. Both processes seem to run their course conjointly but more or less independently of each other, although the degenerative process in the liver tissue naturally facilitates the penetration of it by the connective tissue. There is also much evidence of liver cell regeneration.

The *spleen* shows a marked passive congestion with some thickening of the smaller arteries.

Bacteriologic examination. Smears from the liver contain a moderate number of neutrophilic leucocytes and a moderate number of short bacilli. Examination of sections of the liver stained with Giemsa fails to show any bacteria. Smears from the gallbladder contain no cells, many short bacilli. Culture of the bile shows many colonies of short gram-negative bacilli which are not motile, ferment glucose, coagulate milk, and show a heavy, brown growth on potato (*Bacillus coli*). Smears from the spleen show no bacteria.

Case 3

XXI. 108. E. S., an American housewife, 28 years of age. She entered the San Francisco Hospital with the complaint of pain in the stomach every morning, jaundice and general weakness.

The patient had had the usual diseases of childhood. She was divorced and had had no living children, but six abortions all of which had been induced. She had used beer to excess and had also used considerable whiskey for several years before her death. She had always led a strenuous life of dissipation. She had complained of stomach trouble, intermittently, for the past ten years, consisting of aching pains in the epigastrium in the mornings and after eating. She denied venereal infection.

The present illness had begun with jaundice which had come on suddenly four years before she entered the hospital. It had been associated with severe pains in the epigastrium. She had vomited several times and had passed blood in the stools. The jaundice cleared in four days but the pain persisted. Later hemorrhoids had appeared which at times disappeared for short intervals. The jaundice also had returned and had been complicated by diarrhea. She had lost 15 pounds in weight in six months. She also stated that she had coughed and vomited blood at times.

The clinical examination showed the abdomen distended and tight. The liver was enlarged and anastomoses between the epigastric and mammary veins were evident, but no *caput medusae* could be made out. The urine gave a strong reaction for bile. Blood examination revealed a marked secondary anemia and a slight leucocytosis. The hemoglobin content was 60 per cent. The red cells numbered 2,600,000 per c.mm., the white cells 14,000 with 80 per cent polymorphonuclear neutrophiles.

Clinical diagnoses. Portal cirrhosis; parenchymatous nephritis; aortic stenosis.

Anatomic diagnoses. Alcoholism; cirrhosis of the liver; fatty liver; gastritis, chronic; enteritis, acute; broncho-pneumonia, terminal; salpingitis, chronic; appendicitis, chronic; peritonitis, healed; perisplenitis, chronic; pancreatitis, chronic; jaundice; anemia, secondary.

Abstract of the autopsy record. The body is that of a strongly built woman. The abdomen is slightly distended. The sclerae are icteric; jaundice is marked over the entire body. The peritoneum is smooth. The liver is large, the lower border extending 13 cm. below the xiphoid process in the midline. It extends also fairly well to the left while on the right side it is midway between the costal margin and the crest of the ilium. About 250 c.c. of clear fluid containing some flakes of fibrin are found in the peritoneal cavity. The diaphragm level is at the 4th interspace on both sides. The heart is enlarged slightly, otherwise nor-

mal. The lungs are edematous with beginning broncho-pneumonia at their bases. The spleen is somewhat enlarged (14 x 9 x 6 cm.). The capsule is strongly adherent to the abdominal wall and to the omentum. The kidneys are somewhat swollen and the cut surface is of a grayish-yellow color. The mucous membrane of the stomach is markedly congested and shows a few hemorrhages.

The *liver* is distinctly enlarged measuring 33 x 27 x 10.5 cm. There are fibrinous adhesions over the entire surface of the capsule. The liver is yellow, has a nodular appearance and feels very firm under the knife. The cut surface also shows a nodular appearance with considerable scarring between the nodules.

Histologic examination.

Liver. There are two very striking things in the microscopical appearance of this liver; first, the extreme amount of fatty infiltration; second, the diffuse development of the connective tissue. The latter is greatly increased about the periportal spaces and shows numerous arborizations of finer branches which dissect the lobules or pseudo-lobules producing thereby a very diffuse intralobular fibrosis. As would be expected, the larger cell-groups differ markedly from the true lobules. They vary considerably in size but usually do not attain the size of a normal liver-cell unit. A few lobules show a moderate fibrosis about the centers giving the appearance of a wheel with hub and radiating spokes composed of fibrous tissue (Fig. 5). The trabecular arrangement of the liver cells is almost gone, the cells apparently accommodating themselves to the irregular spaces in the meshwork of connective tissue. The latter is quite cellular and fairly recent. The fibrous tissue is moderately, diffusely infiltrated with round cells; among them are a good many polymorphonuclear leucocytes. In many places small groups of liver cells in the meshes of the new connective tissue show hyaline degeneration and have vacuolate, coarsely granular cytoplasm. They have swollen, pale-staining or very often shrunken nuclei. The process evidently ends in necrosis.

The fatty infiltration is very general. On the other hand in the small groups, or in the periphery of some of the larger ones, are cells with little or no fat. These appear to be normal, active cells. They are relatively few in number and exhibit little tendency to proliferation.

Proliferation of the "bile ducts" is not an outstanding feature of this liver but they are present in a moderate number. Here, as in the preceding cases, apparent transformations between "ducts" and rows of liver cells can be found but they are by no means so plentiful

or so evident as in Cases 2 and 4. There are a few liver cells scattered among the proliferating "ducts" which look like recently formed cells.

The picture presented in this case varies from that in the two preceding cases in the presence of an extreme fatty infiltration and in the absence of any clear evidence of regeneration in the liver cells. In common with the others are the extensive fibrosis, the marked cellular infiltration and the apparently more or less independent, but nevertheless quite general, destruction of liver cells.

Examination of sections of the liver stained with Giemsa fails to show any bacteria.

The *spleen* shows marked passive congestion and some fibrous thickening of the capsule. In the *pancreas* a moderate diffuse, interstitial fibrosis is present with some areas of fat necrosis. The mucous membrane of the *stomach* is moderately infiltrated with round cells and somewhat shrunken in places.

Case 4

XIX. 48. B. C., an Italian dishwasher, a married man of 48 years, entered Lane Hospital complaining of severe pains in his arms and legs, which had lasted for one month.

His general health in the past had been good. He denied venereal infection. He had used alcohol to great excess, stating that he had been drunk most of the time when not working.

The present illness commenced suddenly, one month before entry to the hospital, with pains in the legs and swelling which a week later extended to the arms and the back. His mentality was not clear.

Clinical examination revealed a strongly built, slightly obese man sleeping most of the time. The muscles showed occasional twitchings. Signs of pneumonia were noticed in both lungs. The liver edge was not palpable but there was dullness for 15 cm. below the margin of the ribs in the right mid-clavicular line. Partially healed ulcers and pigmented scars were found on both legs. Both legs were edematous.

The blood picture was that of a moderate secondary anemia with marked anisocytosis and slight poikilocytosis. An occasional nucleated red cell was found in the smears. The hemoglobin content was 84 per cent (Sahli). The red cells numbered 3,100,000 per c.mm., the white cells 10,000. The differential count was normal. The Wassermann reaction was negative in the blood and spinal fluid. A blood culture showed no growth.

While in the hospital the patient showed increasing mental symptoms, arising at night and wandering about the ward resenting any restraint from attendants.

Clinical diagnoses. Delirium, alcoholic; broncho-pneumonia; pyorrhea alveolaris; arteriosclerosis, general with arteriosclerotic kidneys; paraphimosis.

Anatomic diagnoses. Tuberculosis of the lungs, chronic; tuberculosis of the intestines; broncho-pneumonia; pleurisy, chronic; pleurisy, acute; alcoholism, chronic; cirrhosis; balanoposthitis; cystitis, chronic; pyelitis, chronic; pyelonephritis, acute; prostatitis, chronic; conjunctivitis; emphysema.

Abstract of autopsy record. The body is that of a strongly built, muscular, well-nourished man. The conjunctivae are slightly yellow. The feet are somewhat edematous. The abdomen is moderately distended. The peritoneum shows slight passive congestion, but no free fluid is present in the peritoneal cavity. The chest is distinctly barrel-shaped. There are two old cavities in the upper lobe of the right lung. There are also some scattered areas of gray consolidation and some recent tubercles in both lungs. The spleen is slightly enlarged, measuring 17 x 8 x 2.5 cm. Its cut surface is dark red and soft. The gallbladder and the bile ducts are normal.

The *liver* is rather large measuring 30 x 20 x 8 cm. Its tissue is very firm and cuts with considerable difficulty. The cut surface shows numerous small bright yellow spots with gray lines between them. The tissue is especially indurated between the right and left lobes.

Many tuberculous ulcerations are present in the small and large intestines.

Histologic examination.

Liver. The fibrosis in this liver is extensive and in general of the multilobular type. The parenchyma is cut up into irregular patches by the broad bands of fairly old connective tissue (Fig. 7). Throughout the latter one finds a marked cellular infiltration consisting of lymphocytes, a few polymorphonuclear leucocytes and some eosinophiles (Fig. 8). The islands of liver tissue surrounded by this widespread development of connective tissue vary in size. The smaller ones are one-fourth to one-fifth the size of a normal lobule. The larger ones include perhaps two or three lobules or parts of several lobules. The latter are not numerous, however, and usually finer strands of connective tissue dissect these into blocks somewhat smaller than the ordinary hepatic unit.

The liver cells show a moderate fatty infiltration which is quite generally distributed through the sections (Fig. 7). The structure of the lobules is greatly disturbed. The cells have the appearance of being crowded or packed, without any definite arrangement, into the spaces formed by the bands of fibrous tissue. The radial trabecular structure is lacking. The central vein is absent in many lobules and eccentrically placed in others.

Many cells in the smaller and larger groups, and small isolated cell-groups caught in the dense fibrous bands, show hyaline degeneration of the cytoplasm. In others the cytoplasm is swollen, coarsely granular and vacuolated. The nuclei often stain very deeply. Others are swollen, pale, irregular in shape; again others have disappeared completely indicating that many of the cells are undergoing necrosis (Fig. 8).

By far the greater number of cells, however, appear well filled out. Their cytoplasm is finely granular and stains evenly. Their nuclei are round and contain chromatin of normal distribution and staining quality. These are presumably active, functioning cells. Many groups seem to show evidence of regenerative proliferation. Some of these latter cells have large round nuclei and others have two nuclei. While no definite mitoses can be found many of these large nuclei appear to be entering the prophase.

Many proliferating "bile ducts" can be found in and along the edges of the fibrous tissue. The relation of them to the liver cells is interesting. As in Case 2, many seem to be undergoing transformation and various stages may be found between the true "bile-duct" epithelium and typical liver cells.

There is also evident in this case, as in the second one, a certain amount of edema which is discernible in the lobules in places where the cells are not so closely packed.

The general picture in this case seems to be that of an inflammatory condition perhaps of longer duration but less severe than that in the second case. The widespread production of fibrous tissue, the presence in it of cellular infiltration including polymorphonuclear leucocytes, and the presence of edematous fluid all emphasize the distinctly inflammatory character of the lesions in association with a probably toxic degeneration and necrosis of large numbers of liver cells followed by regeneration. The evidence, furthermore, indicates that the process is still progressive.

Examination of sections of the liver stained with Giemsa fails to reveal any bacteria.

Comment

All four patients had taken alcohol to excess for a considerable period of time and two had died with the symptoms of an alcoholic psychosis. Although a discussion of the etiology of cirrhosis is beyond the scope of this paper, we may state in passing that we regard the toxic effect of alcohol as one of the causal factors in this type of cirrhosis. Experimental work, however, points strongly to the existence of other factors which may play an equally essential rôle. The nature of these accessory factors is at present unknown.

Rolleston [5] states that cirrhosis is usually fatal at about 50 years of age. Our cases, as would be expected, since in them the lesions

are relatively early, ended in death at an earlier age than this, the average being 38 years. In the different patients death had occurred at the ages of 39, 38, 28 and 48 years respectively. In none of our cases is death directly chargeable to cirrhosis of the liver, a finding which conforms to Hawkins'[2] observation that early cirrhosis is seen only when death occurs from some other cause. In Case 1 death was due to the rupture of a syphilitic aneurism; in Cases 2 and 3, death was the result, primarily, of the general alcoholic intoxication; while in Case 4 it was caused by the combined effects of tuberculosis (pulmonary and intestinal), broncho-pneumonia and alcoholism.

While most modern writers on cirrhosis are fairly well agreed that the primary lesion in cirrhosis consists in a degeneration of the liver cells which is followed by connective tissue proliferation, our cases seem to show that from the very beginning these two processes occur conjointly and simultaneously. The very early lesions in the liver tissue in Case 1 illustrate this very well. Here we find small foci of liver cell degeneration together with a definitely inflammatory proliferation of the connective tissue (Fig. 2). The inflammatory character of the changes is made evident by the presence of an inflammatory exudate containing polymorphonuclear leucocytes and lymphocytes. In these areas the connective tissue cells are proliferating quite diffusely without close topical relation to the degenerating cells although the advance of the proliferating connective tissue into the liver tissue is evidently favored by the degeneration and necrosis of the liver cells. In our opinion, such lesions as these can be best explained by assuming that the toxin or toxins which injure the hepatic cells in such a way as to cause degeneration ending in necrosis, probably at the same time have an irritating effect upon the more hardy connective tissue, stimulating the connective tissue cells to growth and multiplication and the building up of new fibrous tissue. The reaction would be equivalent to that which occurs so commonly in other forms of proliferative inflammation of infectious or toxic origin.

The distinctly inflammatory nature of the lesions is very evident in all four of our cases. In all of them not only lymphocytes but also polymorphonuclear neutrophilic leucocytes are found in the proliferating connective tissue and about the degenerating liver cells. These are most plentiful in Cases 1 and 2, which are more acute than

the others (see Figs. 2 and 4). In Case 2 they are the predominating type of leucocyte. In addition, signs of an inflammatory edema among the degenerating liver cells and in the new connective tissue are quite evident in Cases 2 and 4.

Leucocytic infiltration, edema and proliferating fibroblasts form the inflammatory part of the process which, however, would not be complete or lead to so much destruction if it were not associated with the degenerative process in the liver cells. The importance of the latter is also clearly shown in all our cases whether they are more acute or more chronic. In all of them microscopic examination reveals much injury to the liver cells resulting in swelling, hyaline and granular degeneration of the cytoplasm, vacuolization and nuclear changes ending in the total disappearance of the nucleus.

The new connective tissue in our cases varies in age from the early fibroblastic stage, in Cases 1, 2 and 4 (Figs. 2, 4 and 8), to that of a fairly cellular fibrous tissue in Cases 3 and 4 (Figs. 5 and 7), but we believe that all four cases still belong to the group of subacute cirrhosis.

Even in these relatively early cases there is abundant evidence of regenerative proliferation of the liver cells. In Case 1 active multiplication of them is clearly shown by the crowding of large numbers of well-preserved cells into the lobules in an irregular fashion (Fig. 1). In Cases 2 and 4 (Figs. 3 and 7) the regenerating cells are found in groups which are more or less circumscribed and which are the forerunners of the hyperplastic nodules so familiar in the hob-nail liver (MacCallum).[6] In our cases these agglomerations are not so large as the nodules commonly found in the late stages of the disease, and since the fibrous tissue around them has not yet contracted, they are less prominent. This explains the relatively smooth surface of the liver in our cases. The evidence of regeneration is less clear in Case 3 than in any of the others.

Many of the earlier writers on cirrhosis have called attention to the increase in the size of the liver in the early stages of the disease. In all four of our cases the liver is above normal size. In Case 1, a woman, the liver is moderately enlarged, measuring 26 x 26 x 7 cm. In Case 3, also a woman, the liver is distinctly increased in size, measuring 33 x 27 x 10.5 cm., while in Cases 2 and 4, who are men, there is a moderate enlargement of the organ, the measurements being 27 x 22 x 10 cm. and 30 x 20 x 8 cm., respectively. Although

there is marked destruction of the liver tissue in all four cases, there are several factors which tend to offset this and which may account for the increased bulk of the liver. These factors are: (1) the increase in young connective tissue; (2) the leucocytic infiltration; (3) the presence of edema; (4) regeneration of liver cells.

Kretz[7] has demonstrated clearly, by means of injected specimens, the profound changes in the blood vessels of the liver which occur in portal cirrhosis. He finds the portal vein dilated, its walls thickened, and its ramifications more numerous than in the normal liver. We find that these changes are fairly well marked even in these early cases. The portal vein is often dilated and its branches are more numerous than in the normal liver. Case 2, which is one of the earlier cases, shows this quite well. On the other hand, according to Kretz, the hepatic veins are reduced in number. This also coincides with our own observations. In many lobules they cannot be found at all while in others they are eccentrically placed.

The development of bile duct-like structures is especially well marked in two of our cases. The relationship of these new "ducts" to the liver cells is a very interesting one, but the controversy over the part which they play in the regeneration of liver tissue is still to be decided.

When, as pointed out in the records, the proliferating "bile-ducts" are occasionally seen to end in club-shaped groups of liver cells with a transitional type of cell connecting them, it is natural to assume that the "bile ducts" are undergoing transformation into rows of liver cells. This assumption is strengthened by the presence of regenerated liver cells in the vicinity; and by the fact that embryologically the liver cells develop from the epithelium of the primitive bile ducts. The proliferating "ducts" are usually found in the new connective tissue often near or scattered among hepatic cells which have the appearance of being recently formed. Furthermore, the "ducts" are not found in the regions where the liver cells are undergoing destruction. These observations suggest that the "bile ducts" function in the process of regeneration. The appearances pointing to the transformation of "bile ducts" into liver cells is very marked in Cases 2 and 4. In Case 2, especially, many "bile ducts" can be found which terminate in rows of liver cells which have every appearance of being recently formed. Our findings coincide with the observations of many of the prominent investigators of regeneration

of the liver such as Waldeyer (1868), Hanot (1895), Stroebe (1897), Rolleston (1905), Meder (1905), and MacCallum (1904). It may be pointed out however that such interpretation of the development of one type of cell into another from the presence of "transitional" stages is always open to criticism.

On the other hand, some of the "bile ducts" are narrow, flattened strands of cells which appear to have been mechanically compressed. These evidently show that some of the "bile ducts" arise from atrophied rows of liver cells rather than the reverse.

Conclusions

1. Histologic study of relatively early, progressive cases of alcoholic cirrhosis reveals the inflammatory nature of the changes in the connective tissue by the presence of the usual signs of a subacute proliferative inflammation, namely, inflammatory edema, infiltration with leucocytes, among them many polymorphonuclear neutrophiles, and proliferation of the connective tissue cells.
2. Evidence of injury to the liver cells is always present but apparently does not precede the proliferation of the connective tissue. The two processes occur simultaneously, probably as a result of the action of a common cause.
3. The characteristic hyaline degeneration of the cytoplasm of the hepatic cells, pointed out by Mallory as peculiar to alcoholic cirrhosis, was found in all our cases.
4. The history of all four patients revealed chronic alcoholism and alcoholism was the principal cause of death in two of them.
5. Regeneration of liver cells may be a prominent feature even in early cirrhosis.
6. In all our cases the liver was somewhat larger than normal, in one of them it was considerably enlarged.

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DESCRIPTION OF PLATES LXXVIII-LXXXI

FIG. 1 (Case 1). Low power view of the liver showing patchy distribution of the connective tissue, cellular infiltration, fatty infiltration and pale-staining liver cells (glycogen). The cells are crowded and the normal arrangement of the lobule is disturbed due to rapid regeneration of liver cells.

FIG. 2 (Case 1). High power view of early focal lesion, showing destruction of liver cells with hyaline and granular degeneration of the cytoplasm, pyknosis and disappearance of nuclei, marked infiltration with polymorphonuclear leucocytes and lymphocytes, active proliferation of connective tissue cells.

FIG. 3 (Case 2). Low power view showing widespread destruction of liver tissue, extensive production of new connective tissue and marked cellular infiltration, many new "bile ducts" in the connective tissue. At the lower edge is a group of regenerating liver cells.

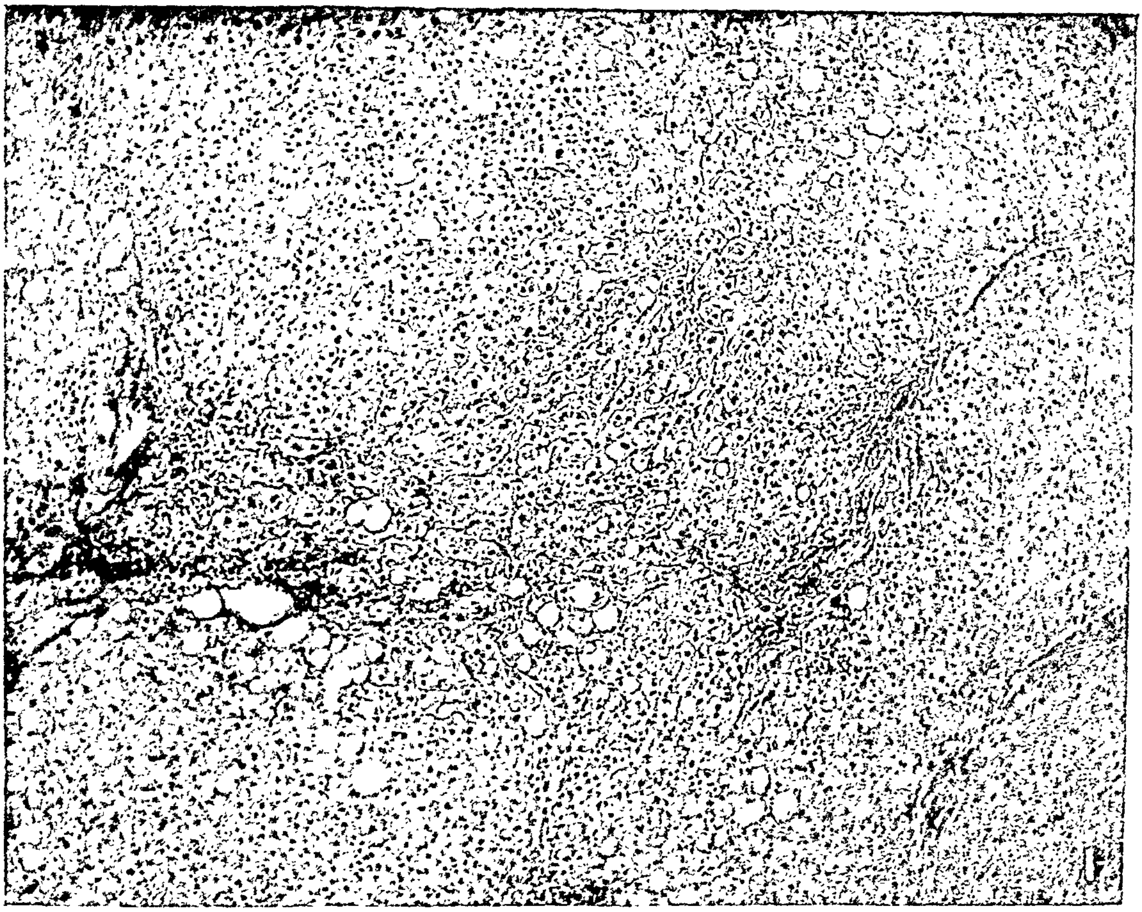
FIG. 4 (Case 2). High power view showing destruction of liver cells. The cells are swollen, several show hyaline and granular degeneration of the cytoplasm with marked vacuolization in some. Several cells are almost completely destroyed. The nuclei of the degenerated cells are shrunken, pyknotic, some have completely disappeared. The new connective tissue is heavily infiltrated with polymorphonuclear leucocytes.

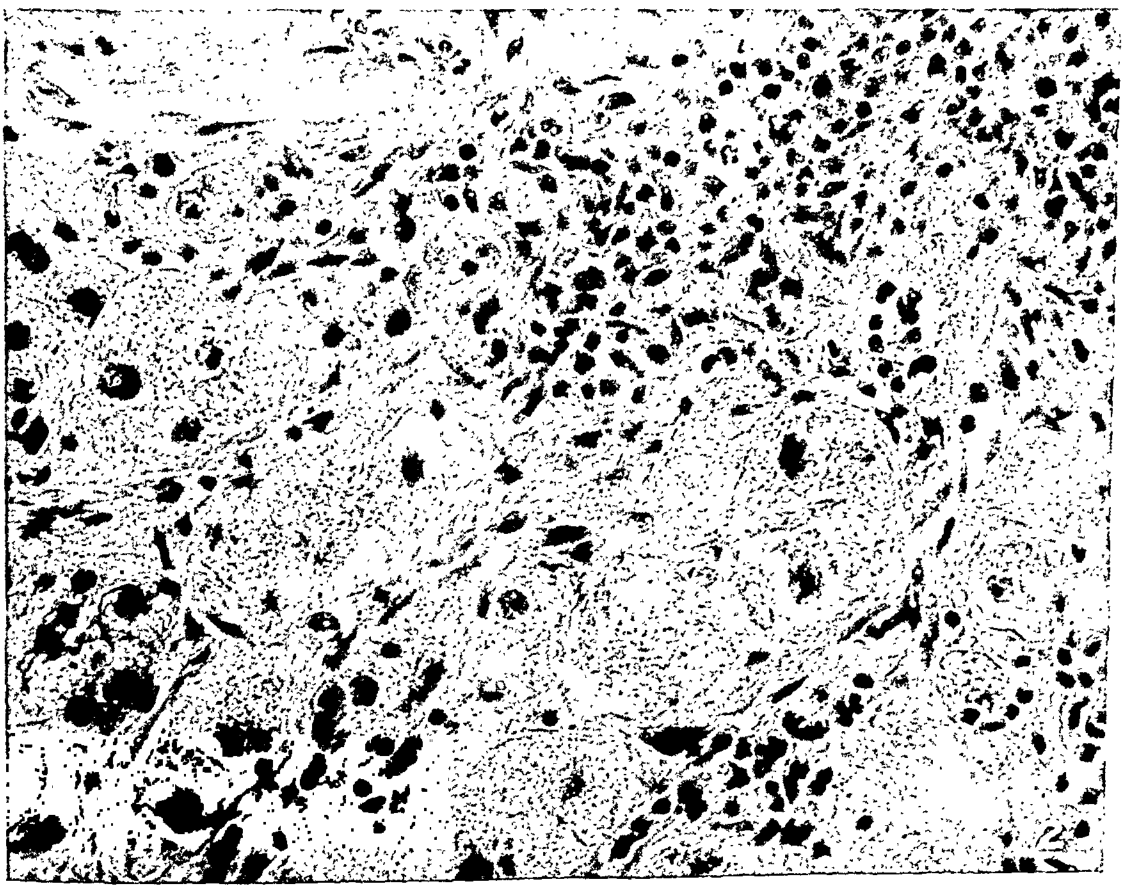
FIG. 5 (Case 3). Low power view showing the peculiar distribution of the new connective tissue and the extensive fatty infiltration of the liver cells, also the marked cellular infiltration of the fibrous tissue.

FIG. 6 (Case 3). High power view showing marked destruction of liver cells with granular and hyaline degeneration and vacuolization of the cytoplasm. Several cells show large globules of fat in the cytoplasm. Proliferation of the connective tissue can be seen among the degenerating cells; also infiltration with polymorphonuclear leucocytes and lymphocytes.

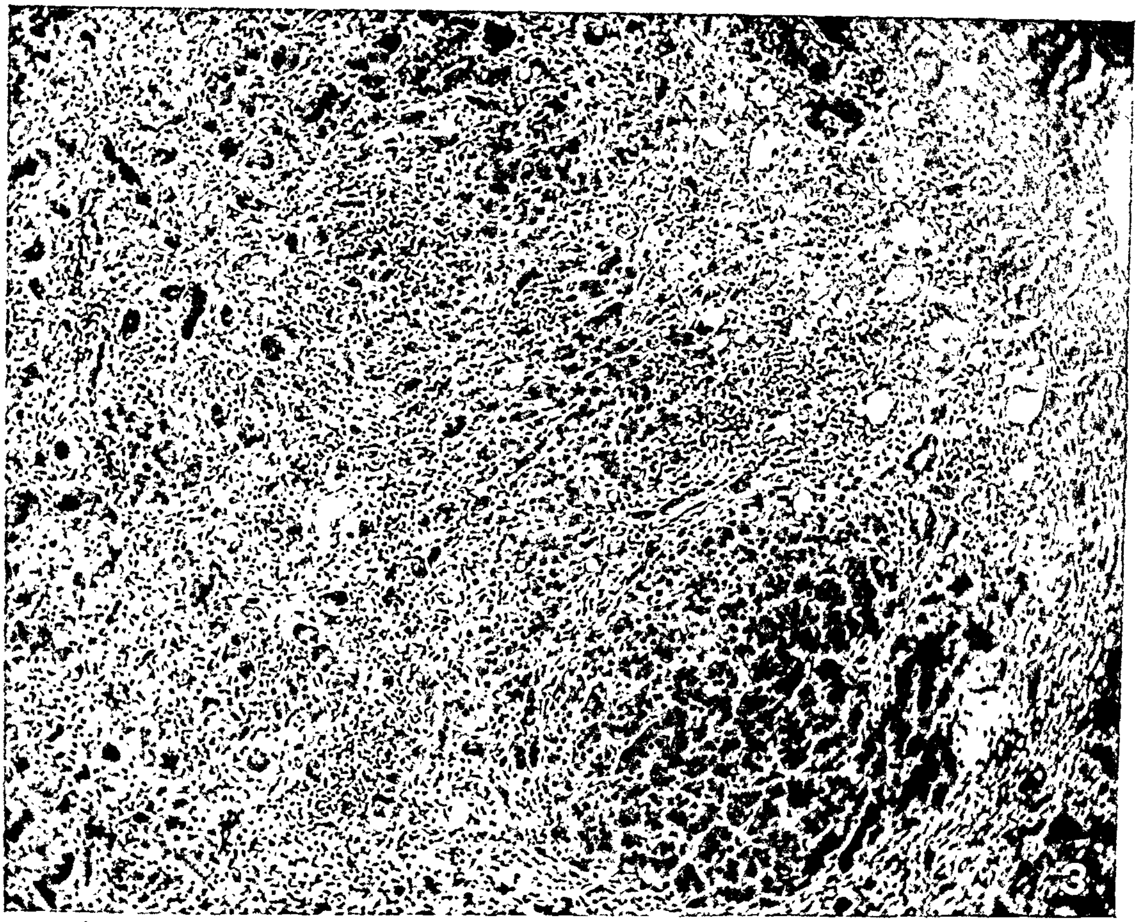
FIG. 7 (Case 4). Low power view showing distribution of the abundant fibrous tissue with very heavy cellular infiltration. There is some fatty infiltration and much evidence of regeneration of liver cells.

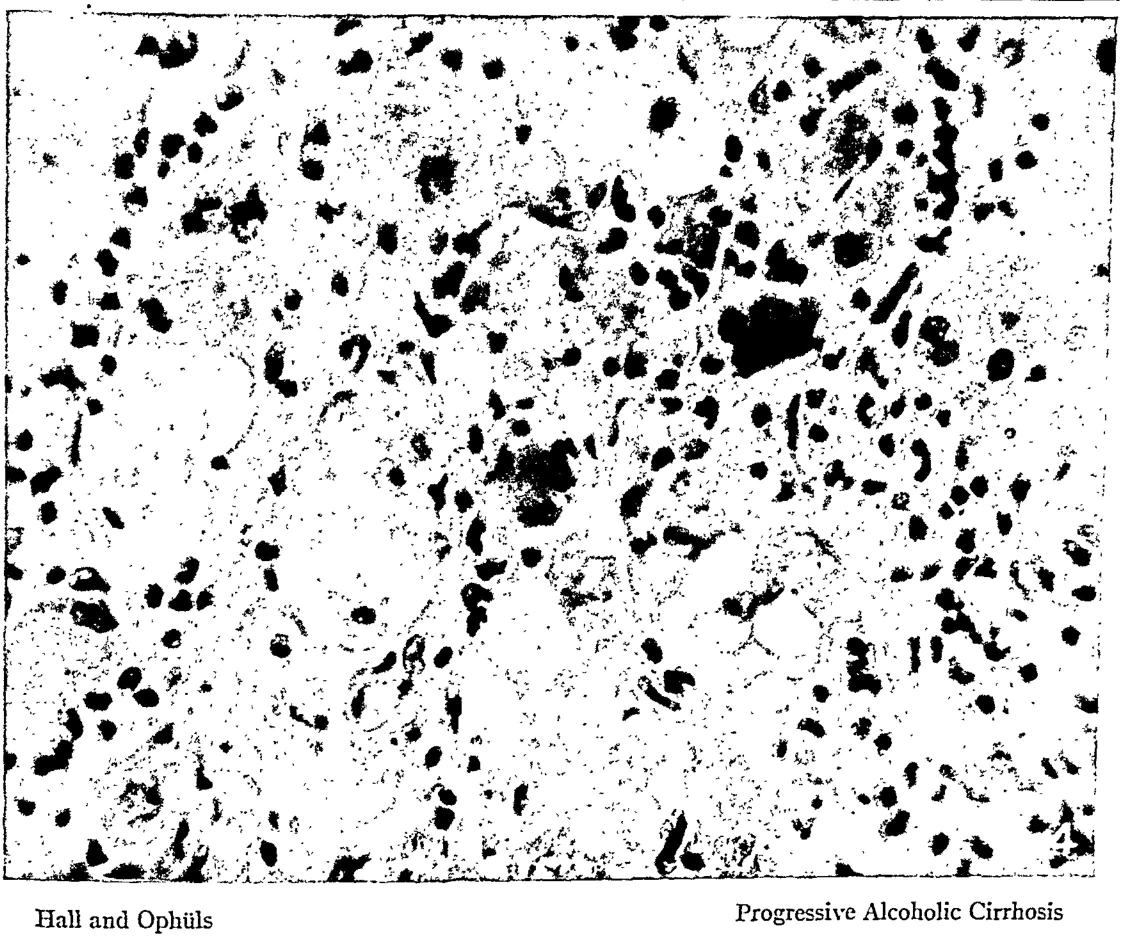
FIG. 8 (Case 4). High power view showing destruction of the liver cells with extensive hyaline and granular degeneration of the cytoplasm. The new connective tissue is heavily infiltrated with polymorphonuclear leucocytes and lymphocytes.





Hall and Ophüls Progressive Alcoholic Cirrhosis





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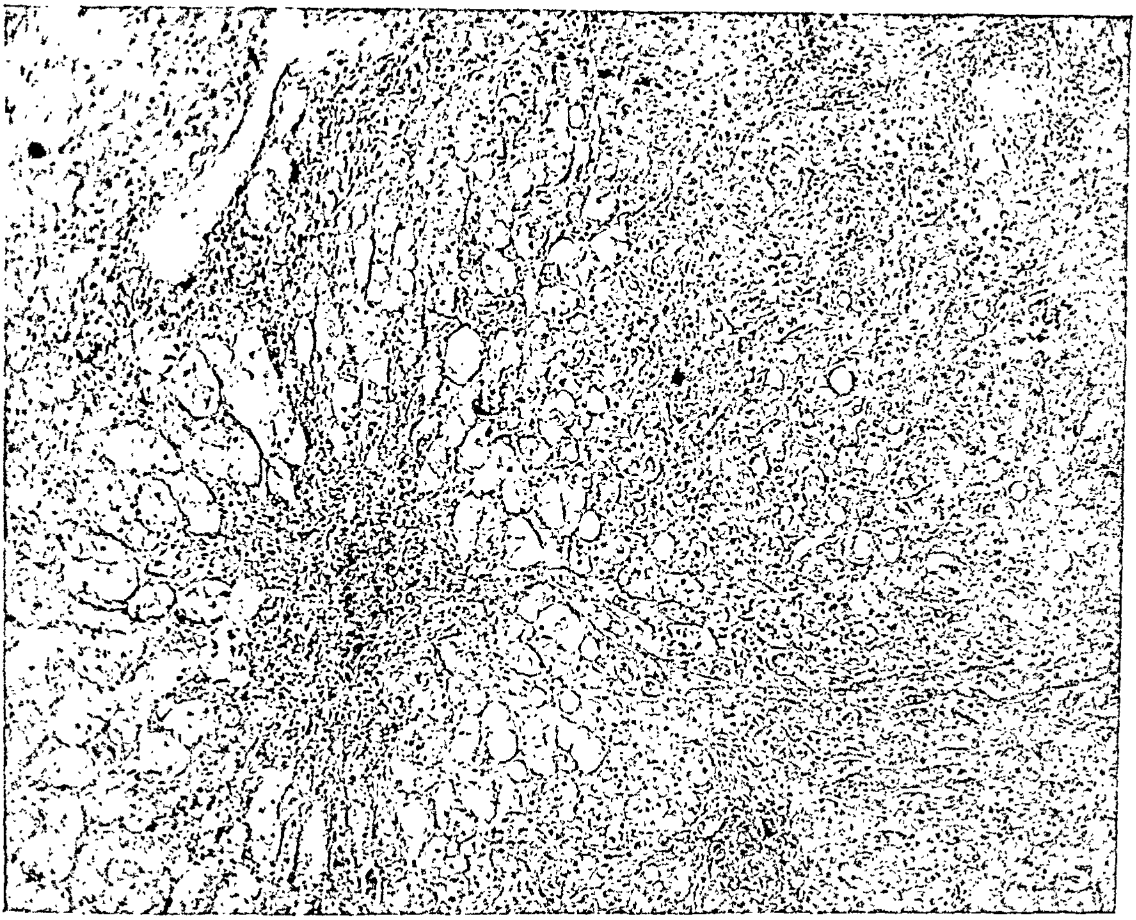
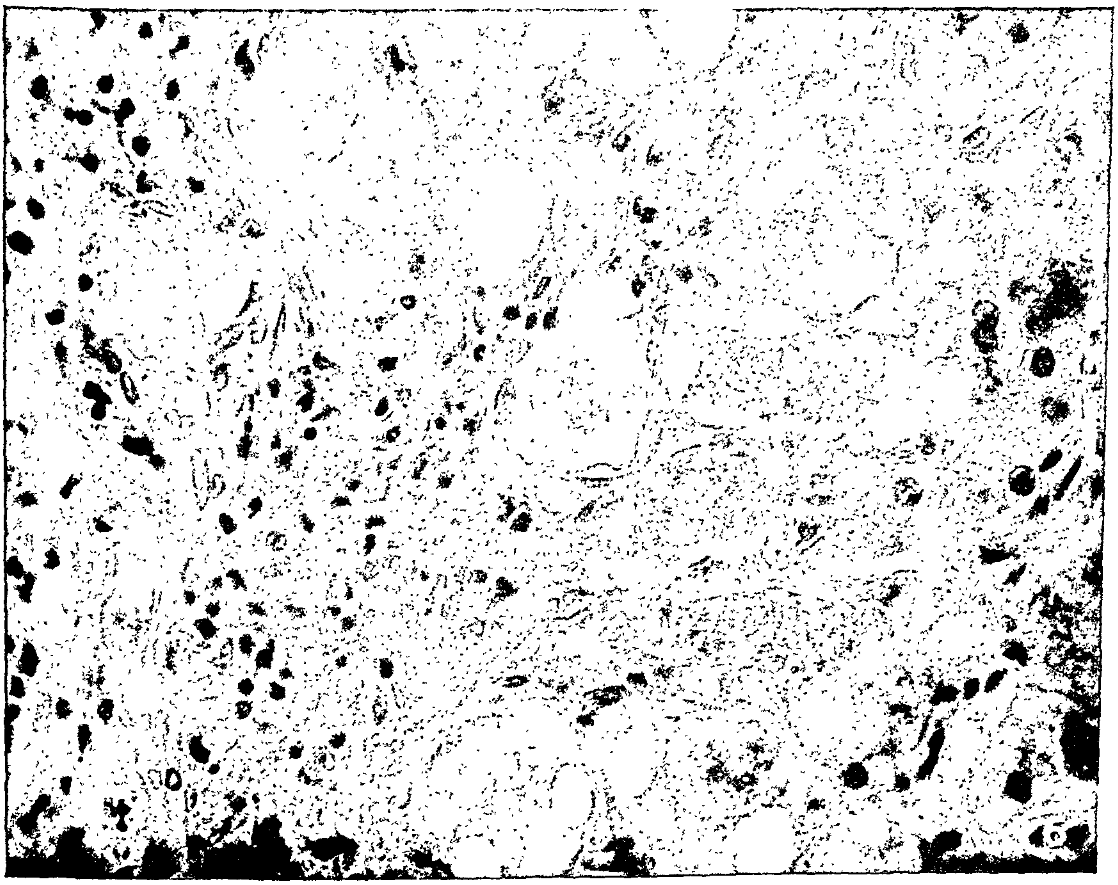
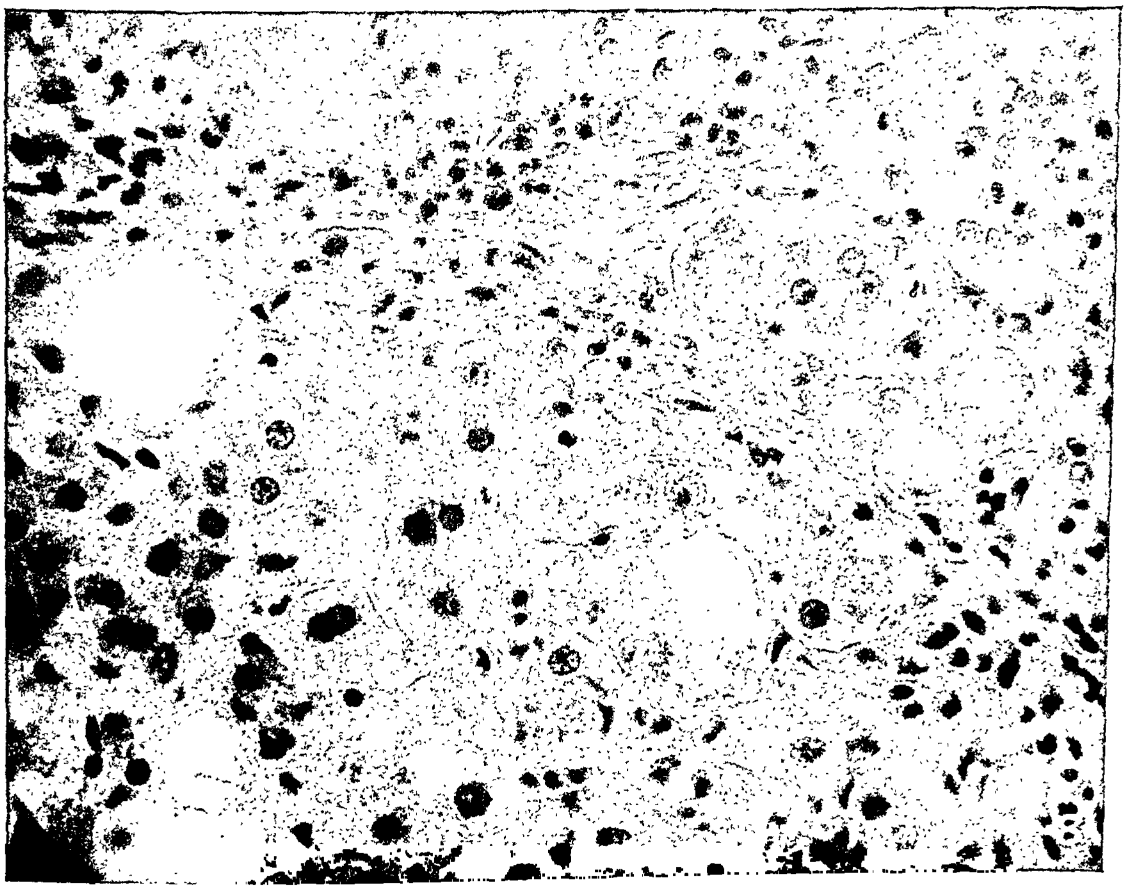




Hall and Ophüls

Progressive Alcoholic Cirrhosis



Hall and Ophüls

Progressive Alcoholic Cirrhosis

OBSERVATIONS ON FOCAL TUBERCULIN HYPERSENSITIVENESS IN AN INFECTED ORGAN PREVIOUS TO A GENERAL SENSITIZATION *

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In a previous study [1] concerned with allergic reactions in testes of guinea-pigs sensitized by treatment with killed tubercle bacilli, the observation was made that definite attempts at healing occurred in testes of untreated control animals before these animals had become generally hypersensitive. By "healing" is meant especially fibroblastic proliferation, though of course healing in tuberculosis includes much more than this. The untreated control animal reacted to testicular infection with the B–I strain of tubercle bacillus with fibrosis before the pig had become generally sensitized by his infection or at least before an intradermal tuberculin reaction had been positive.

This fact raised a question as to whether or not an animal with testicular infection would react to tuberculin introduced into the infected organ before the animal gave a positive intradermal reaction to like dilution; in other words, is a local sensitivity to tuberculin demonstrable in an infected organ before fibroblastic proliferation, as evidence of walling-off or "healing," becomes evident? To test this question the following experiment was devised. Seven normal guinea-pigs, each weighing over 600 gm., were given 0.1 c.c. of a saline suspension containing 2.5 mgm. per c.c. of living bovine tubercle bacilli. The B–I strain was used and the right testis infected. On the second, third, fourth, fifth, sixth, seventh and eleventh day after infection, 0.1 c.c. of 5 per cent old tuberculin was injected both into the infected and into the normal testis, and a like quantity was given intradermally. Animals were killed about eighteen hours after testing. In a second series of five pigs both testes were infected with similar doses of the same bovine strain; two, four, six, seven and eight days later one testis was tested with 5 per cent tuberculin, the intradermal test was done, and the second testis received 0.1 c.c.

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of physiological saline. In a single uninfected animal, one testis was injected with 5 per cent tuberculin, the other with physiological saline. Again, animals were killed eighteen hours after testing. Testes were fixed in Zenker's fluid, cut at 6 microns and stained in eosin-methylene blue. Some were fixed in alcohol-formalin, embedded in celloidin and stained with hematoxylin and eosin.

It may be stated at the outset that definite focal reactions to tuberculin occurred in the infected organ before a positive skin test was obtained. This brings up the question as to what constitutes a positive intradermal reaction. Tuberculin is not innocuous to the normal animal. The author has read upwards of 1000 intradermal tests on guinea-pigs and has frequently observed in normal animals transient redness or transient induration persisting twenty-four to forty-eight hours after intradermal tuberculin and has occasionally carefully autopsied an animal giving a questionable positive skin test, always with negative results as to the presence of infection. Simple blanching of the skin likewise is common, possibly being the rule in an unsensitized animal. The combination, however, of central blanching, with surrounding erythema and induration, even if slight, is always significant, and in the writer's recollection has almost always meant sensitization or infection verifiable either by autopsy or by other methods. One exception to this rule was the case of a female guinea-pig confined with a male sensitized by killed tubercle bacilli injected into the testis. This pig gave several very suggestive reactions, with blanching, induration and erythema but sensitization was never verified; there have been other rare instances. In the present study a "positive" intradermal reaction refers to one with at least central blanching, peripheral erythema and induration.

Some reaction is produced by injection of 5 per cent old tuberculin in the testis of a normal guinea-pig. Eighteen hours after such injection histologic examination shows essentially normal tubules, with slight evidence of desquamation or cessation of spermatogenetic activity. Between the tubules, however, there is a moderate fibrinous exudate (Fig. 6) and numerous polymorphonuclear leucocytes and eosinophiles are present. Lymphocytes are rare but a moderate number of phagocytic endothelial leucocytes occur. These latter seem highly phagocytic for polymorphonuclears. Changes induced by tuberculin in the normal testis are diffuse and affect the entire organ.

Physiological saline in the normal testis produces no definite alteration within eighteen hours, although an occasional extravascular lymphocyte may be found. Traces of fibrin present are probably due to trauma.

The first pig of Series I, infected in the right testis, received 0.1 c.c. of 5 per cent old tuberculin, forty-eight hours later, in each testis, and a like quantity intradermally. Eighteen hours afterward the intradermal test was negative. Grossly the right testis was more injected than the control and the tunicae were non-irritable; that is to say, the fine tremors seen in the muscle after death and exposure to air currents were lacking. Microscopically, however, aside from a small extravasation of blood at the point of entry of the needle, there was nothing beyond what might be expected from tuberculin injected in a normal testis, save for a certain increased amount of desquamation of epithelium and infiltration by endothelials, easily explainable on the basis of infection alone. At least it may be stated that there was no focal change such as we shall see occurs after tuberculin in animals subsequently described. The opposite uninfected testis shows a polymorphonuclear infiltration and traces of desquamation not exceeding that seen in testes of normal tuberculin-injected guinea-pigs. There was no focal lesion. In Pig II, tested in the same manner as the preceding, but twenty-four hours later, in addition to polymorphonuclear infiltration, and the usual evidences of tuberculous infection, there are a small number of tubules which show much more marked desquamation, actual necrosis and extensive invasion of the epithelium and of the tubular lumen by polymorphonuclears. In addition there is considerable subcapsular fibrin, some hemorrhage possibly due to the trauma of injection, and much polymorphonuclear reaction beneath the capsule. In other words there are focal changes; their significance is only determinable in the light of later stages. The skin test and the opposite tuberculin-injected testis of Pig II were not remarkable.

Pig III received tuberculin into each testis and intradermally four days after testicular infection. Eighteen hours later the skin showed a slight atypical blanching with neither surrounding erythema nor induration, a test interpreted as a frank negative. The control testis exhibits possibly a trace more reaction than one might expect in an unsensitized animal but too little to be certain. Changes likewise are not focal. The infected testis, however, in addition to

the general changes due to infection shows a well-marked focus of increased desquamation and necrosis of tubules with marked surrounding polymorphonuclear infiltration and invasion of pink-staining necrotic tubules by polymorphonuclear leucocytes. These changes are certainly to be interpreted as reaction. Unfortunately the sections are from celloidin-embedded material and good photographs could not be obtained. Pig IV received identical treatment five days after infection, and was killed on the sixth day. The skin showed simply blanching without erythema or induration. The control testis microscopically is negative save for the non-specific tuberculin reaction (Fig. 5) and evidences of reaction to the trauma of needle puncture. Figure 1 gives some idea of the histology in the infected tuberculin tested testis. The lower half of the figure, approximately, shows the appearance of the infected testis. The tubules show no evidence of spermatogenesis. The epithelium is desquamated and elsewhere in the organ the lumina of tubules contain large epithelial giant cells formed either by fusion or multiple division of epithelial cells. Other tubules show large vacuolated cells. Surrounding these tubules the reaction is mainly of the endothelial and lymphocytic type (Fig. 7). Polymorphonuclear leucocytes and eosinophiles are rare. There is a heavy deposit of fibrin in the subcapsular region and between testis and epididymis, and large numbers of extravascular red cells are present. The upper portion of the figure possesses an entirely different appearance. The tubules exhibit a degree of necrosis far beyond a possibility of adequate demonstration by photomicrograph. Epithelial cells are almost wholly desquamated (Fig. 4); they take a deep pink eosin stain characteristic of necrotic tissue. Nuclei are largely absent and surrounding almost every tubule are dense collections of polymorphonuclear leucocytes. Curiously enough in these early tuberculin reactions edema is lacking or is present only in traces. Grossly the infected testis of Pig IV was no larger than the control and neither was larger than the normal organ. The infected testis differed grossly from the control only in degree of redness, the size of dilated venules and in the appearance of the tunicae; the latter in the infected testis were loosely adherent to the organ. In all early stages necrosis is the marked distinguishing feature, and had it not been for histologic examination this would not have been apparent and the testis would probably have been noted as non-reacting.

Pig V, tuberculin-injected as were the preceding, on the sixth day and killed eighteen hours later showed a negative intradermal test. In the infected testis, however, the necrotic tissue was very evident in gross; the testis presented an area of marked pallor with surrounding erythema resembling features characteristic of an early positive intradermal test. The control testis was not remarkable. Histologically (Fig. 2) the infected testis resembles that of Pig IV, a typical progressive tuberculosis with much endothelial cell infiltration, lymphocytes, plasma cells and rare polymorphonuclears. Beside this there is a sharply outlined necrotic zone, with pink-staining tubules filled with non-nucleated, desquamated epithelium and surrounded by dense masses of polymorphonuclears. For the first time the control testis shows evidence of reaction, similar to that found in the infected testis. There is a very minute focus where typical necrosis of tubules with surrounding polymorphonuclear leucocytes stamps the organ as a reacting structure. Edema is absent.

Pig VI, twenty-four hours later, had a negative intradermal test. The infected testis reacted similarly to the testes of earlier animals, and its histologic appearance (Fig. 3) is almost identical with them. The uninfected control testis shows a small focal reaction, no more than did the previous control.

The remaining animal was kept until the eleventh day after infection before being similarly treated with tuberculin. It was felt necessary to be certain that a positive intradermal test would eventually be obtained. Eighteen hours after the usual injection the intradermal test was definitely positive although not a strong positive. There was central blanching with a faint bluish tint, edema, surrounding erythema and induration, but no eschar. Both testes, however, were enlarged to twice normal size, were reddened, firm and edematous. The spermatic cord was injected and edematous and there was blood-tinged fluid in the peritoneal cavity. Liver, spleen and retroperitoneal nodes showed tubercles. Microscopically both testes exhibit the usual typical reactions of the sensitized testis, — necrosis, polymorphonuclear infiltration and here for the first time there is extensive edema.

The second series of pigs was used to eliminate the factor of trauma, since it was not certain that the necrosis observed after tuberculin injection in an infected organ was not traumatic. Therefore,

both testes were infected each with the same dosage of organisms as that used in Series I, doubling consequently the total infecting dose. Then at stated intervals, one testis and the skin received each 0.1 c.c. of 5 per cent old tuberculin and the second testis was injected with 0.1 c.c. physiological saline. Forty-eight hours after infection the first pig was treated in the fashion just described. The skin test was negative, the saline-injected testis is negative histologically save for the early evidences of infection and the tuberculin-injected testis shows no change which may be interpreted as due to sensitization. The second animal, tuberculin-treated at ninety-six hours and killed eighteen hours later, gave a negative skin test and its testes show no focal changes microscopically save those of infection. At 144 hours the pig showed a skin test interpreted as a weak positive; the saline-injected testis shows no reaction histologically but the tuberculin treated testis exhibits a weak reaction. This pig has been the one exception in the two series and it may be that in some manner it received a larger infecting dose and reacted generally earlier than did others of the series. Or, more likely, since the focal testicular reaction is weak, it may be that the intradermal test was one of the rare false positives. The two remaining animals of Series II injected at 168 and 192 hours both gave negative intradermal reactions but reacted focally in the infected testis in the same manner as did animals of the first series.

Conclusions

1. The guinea-pig testis infected with bovine tubercle bacilli reacts focally to 5 per cent old tuberculin before the opposite uninfected testis shows a positive reaction and before a positive intradermal test is demonstrable.
2. This focal reaction is characterized by a sharply demarcated zone of increased necrosis of tubules surrounded by a dense exudate of polymorphonuclear leucocytes.
3. Physiological saline in an infected testis will not give a similar picture.
4. The significance of this earlier focal hypersensitiveness in an infected organ is wholly problematical. It probably means merely a more intense local flooding with antigen, but can have no bearing on the question of local origin of antibodies or of local immunity.

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DESCRIPTION OF PLATES LXXXII-LXXXV

Fig. 1. Infected testis, tuberculin-injected on the fifth day. Necrotic tubules occupying approximately the upper half of the figure. Dense exudate of polymorphonuclears surrounding the necrotic tubules. X 100.

Fig. 2. Infected testis, tuberculin-injected on the sixth day. Necrotic tubules in the upper half of the figure, polymorphonuclears forming a sharp boundary between reacting and non-reacting portions of the testis. X 100.

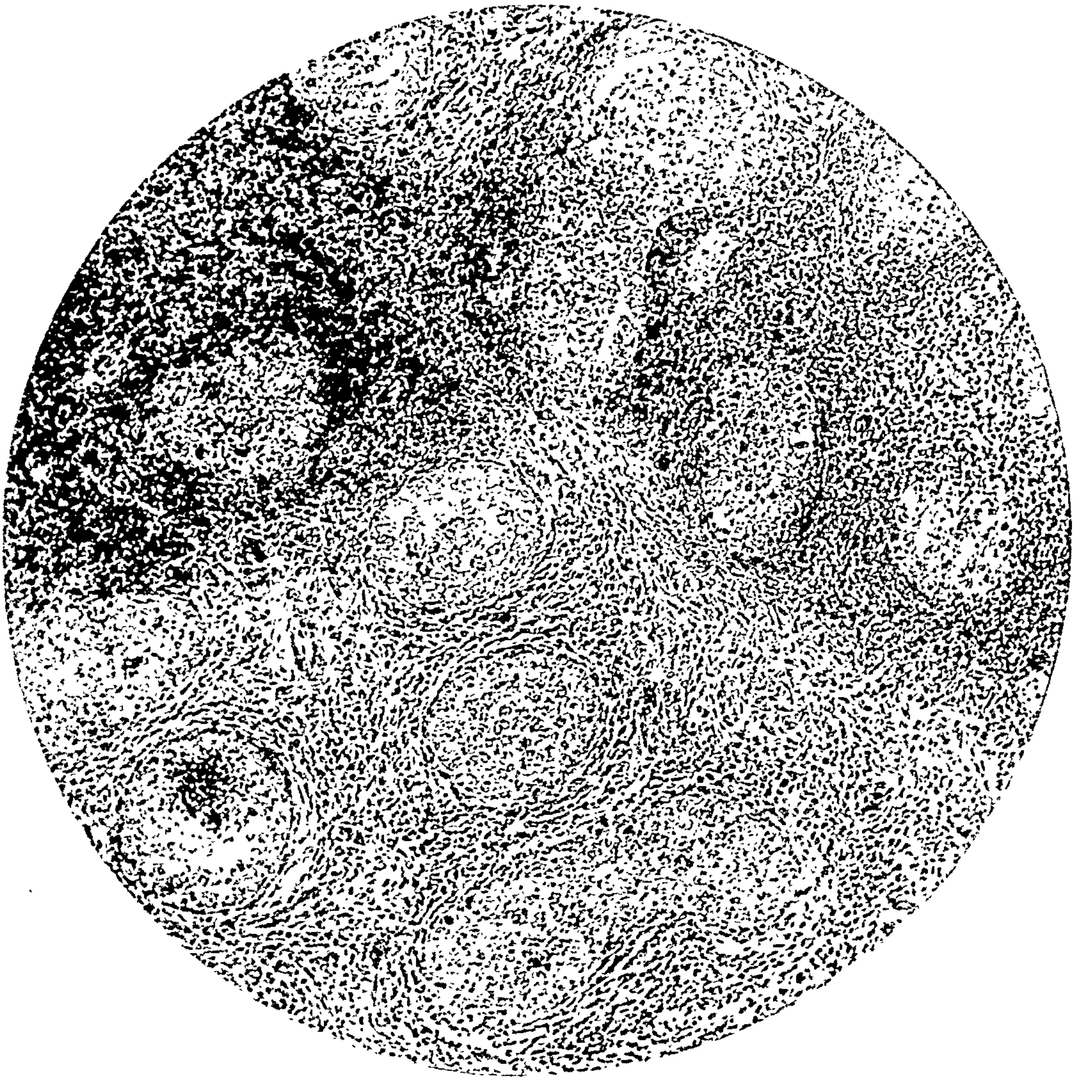
Fig. 3. Infected testis, tuberculin-injected on the seventh day. The usual sharp line of polymorphonuclears separating necrotic from non-necrotic tubules. X 100.

Fig. 4. Necrotic tubules and polymorphonuclear reaction in infected testis, tuberculin-injected on the fifth day. X 250.

Fig. 5. The opposite uninfected testis of the same animal. This testis received the same dosage of tuberculin. X 200.

Fig. 6. Showing the effect of tuberculin in the testis of an uninfected animal. X 200.

Fig. 7. Infection alone: the zone outside of the reacting tissue, infected testis, tuberculin-injected on the fifth day. X 250.



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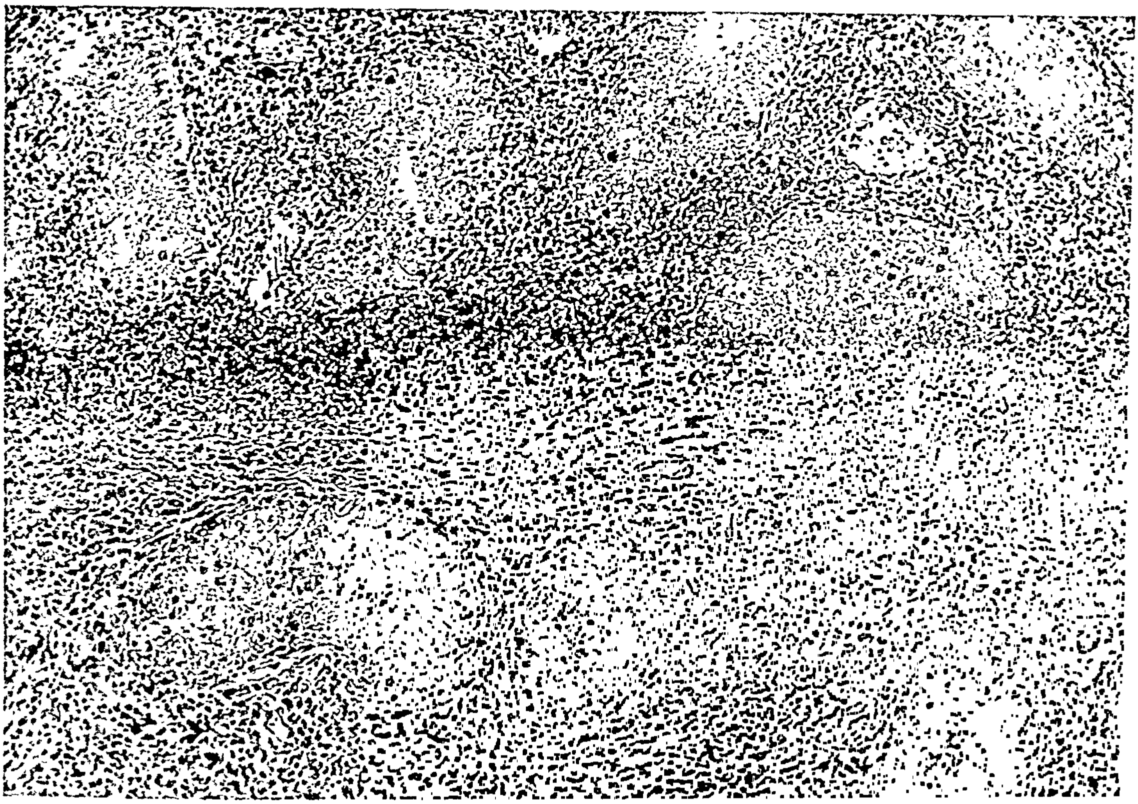
Stewart

Focal Tuberculin Hypersensitiveness



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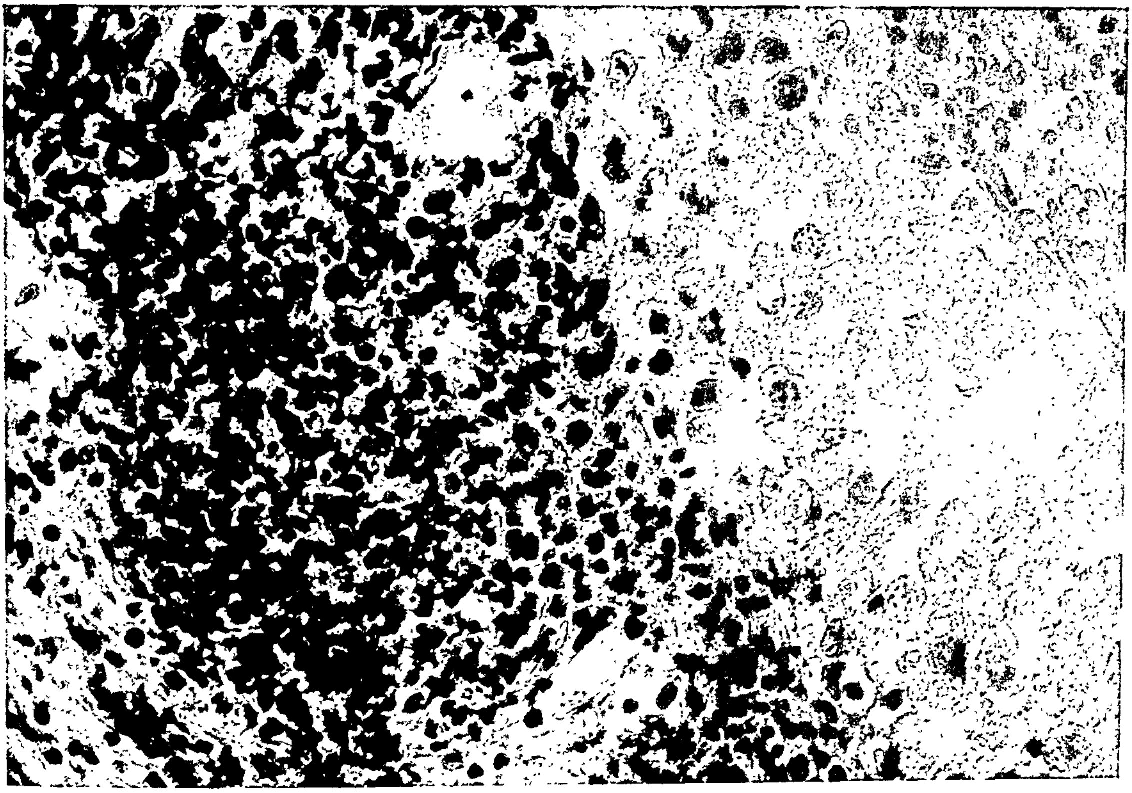

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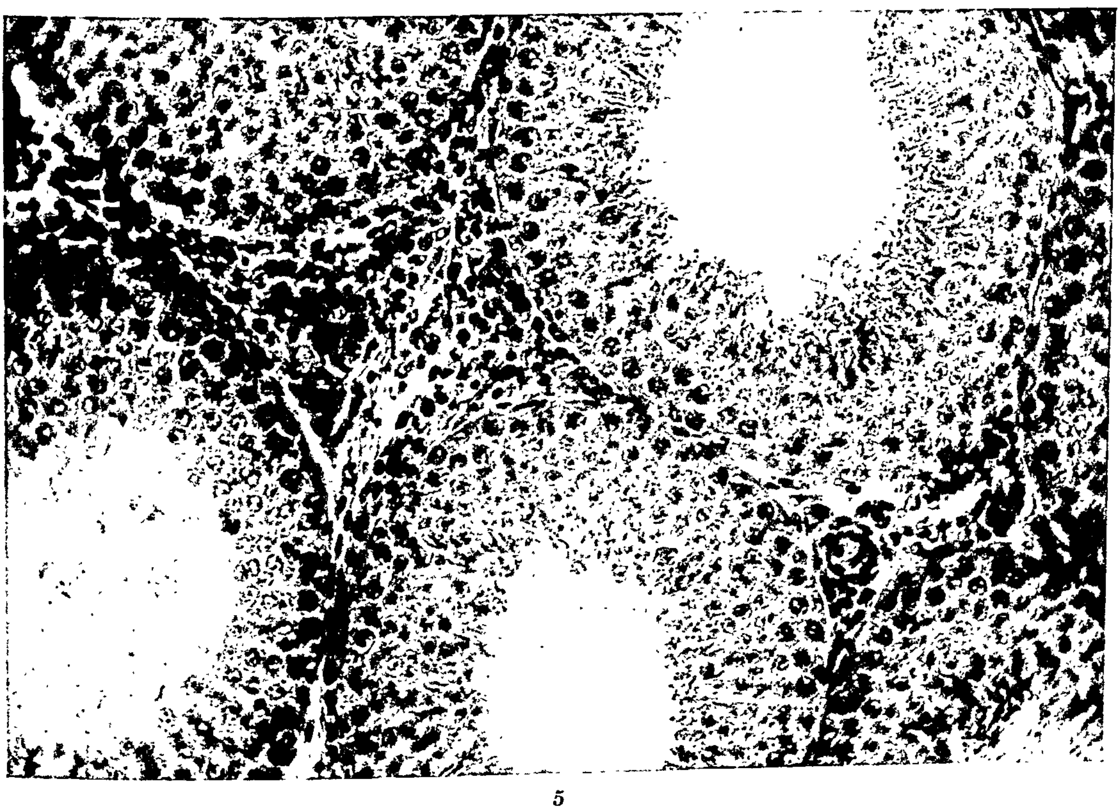

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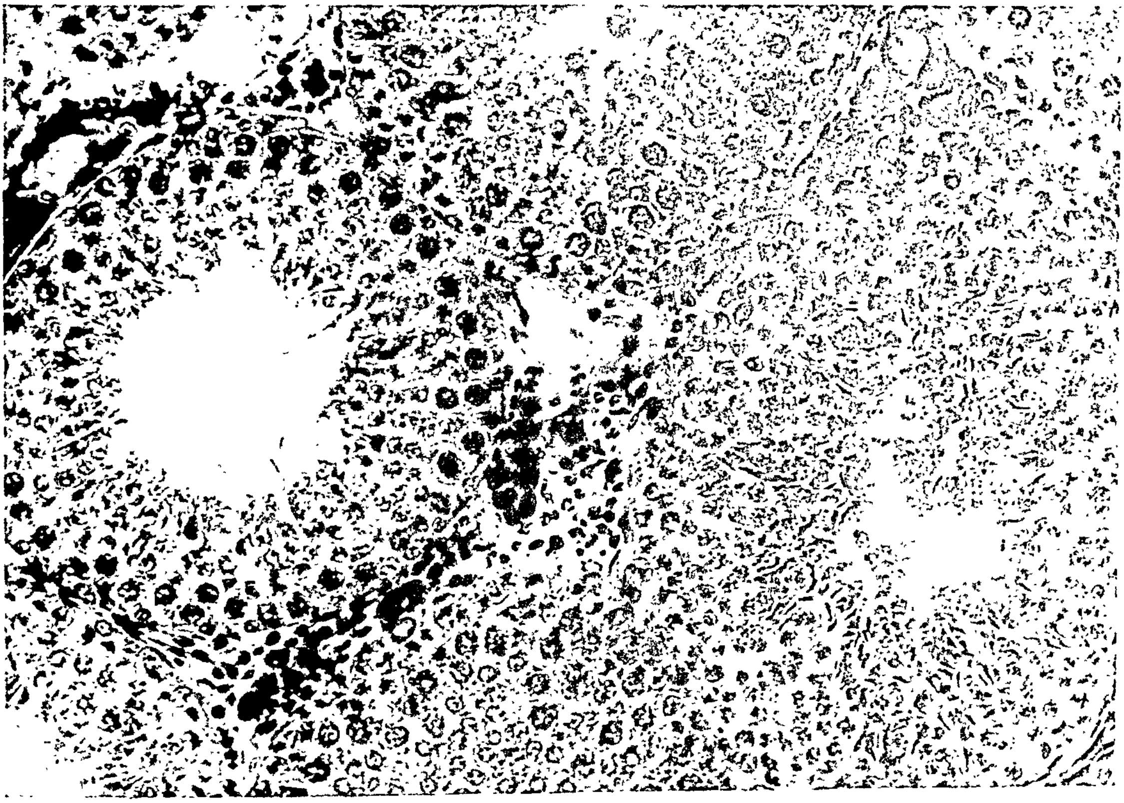

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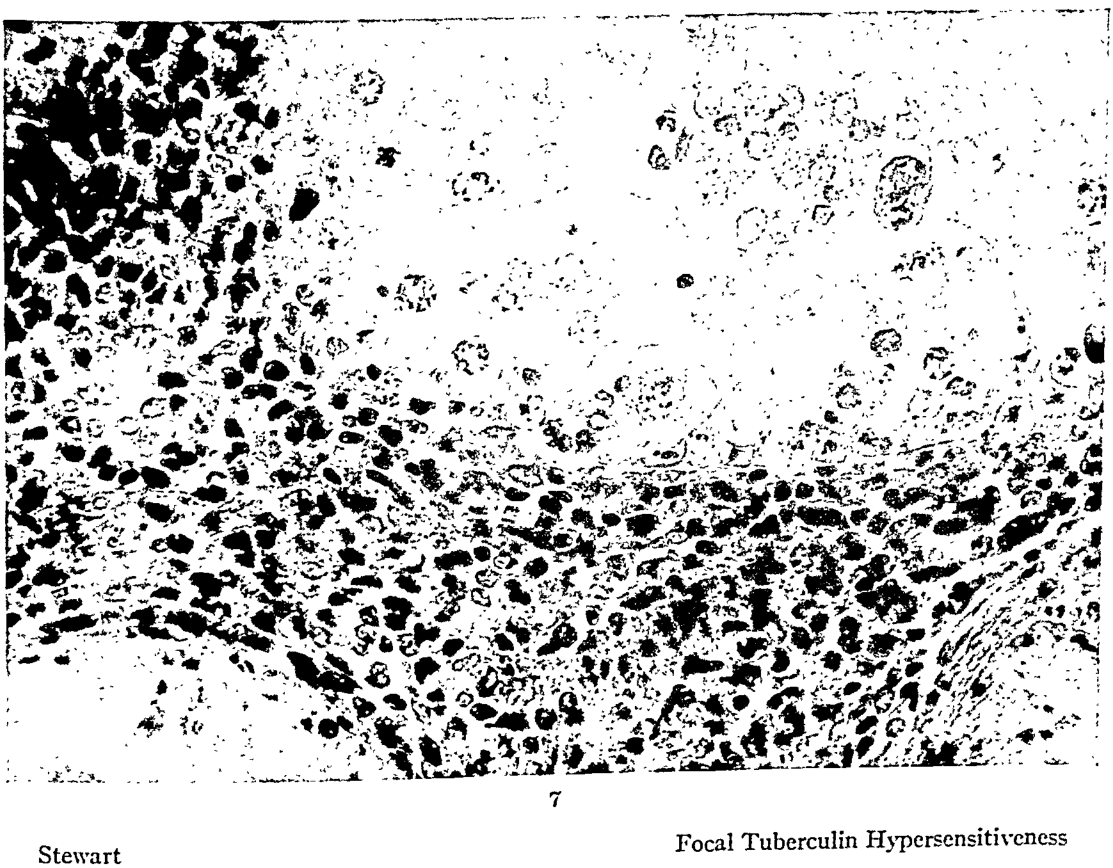

7

GROSS AND HISTOLOGIC CHANGES IN THE EDEMA OF PARAPHENYLENEDIAMINE *

M. L. Tainter and E. M. Hall

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The striking edema of the head and neck regions appearing in about one and one-half hours after administration of paraphenylenediamine to rabbits involves mainly the tongue, conjunctiva, skin of the face and lips, submandibular tissues, larynx, and vocal cords. Anatomically, therefore, its location appears to be rather specific. Physiologically, the mechanism of its formation is concerned essentially with a marked increase in vascular permeability.[1] The response of cats to paraphenylenediamine [2] is similar to that of rabbits, except that edema of the larynx and vocal cords occurs more markedly and rapidly in cats than in rabbits. Studies of this experimental edema may ultimately have fundamental and practical bearings, especially in view of the occurrence of specific edema processes [3] in other regions from another compound (metaphenylenediamine) differing from paraphenylenediamine by the position of an amino group only.

As far as we know, histologic studies of the interesting edema of paraphenylenediamine have not been made previously. It is, therefore, the object of this paper to report the changes observed in ten rabbits with the desire of completing a study of various phases of the phenomenon by one of us (M. L. T.), and of demonstrating that the edema is specifically localized. Before discussing the histologic changes, a short summary of the gross changes at necropsy will be presented. This has been made from a careful study of the changes in over 350 rabbits and cats by one of us (M. L.T.).

Changes at Necropsy

At necropsy there was a variable amount of chemosis in the conjunctivae without hyperemia or signs of irritation. The skin of the face was usually edematous, the nose being about twice its normal

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width. The tongue was always markedly swollen, and on section appeared soggy and gelatinous. Frequently the tissues of the floor of the mouth were raised into large gelatinous bullae. In the neck, the edema was confined mainly to the area between the rami of the mandible, which was filled with a large collection of semi-gelatinous and translucent material. The gelatinous consistency of this material was due to the presence of fibrin, since it was previously demonstrated[1] that whole plasma with its fibrinogen escaped into the area of edema. The vocal cords were often in tight apposition because of the bullous swelling present, which at times extended to the mucosa and muscles of the larynx. The muscles of the head and neck frequently appeared blackened, as if stained by the drug or some changed form of it. This discoloration was never observed in other muscles of the body, except at the site of the injection where the paraphenylenediamine came into direct contact with the underlying musculature. The selective staining of the head and neck muscles may be regarded as further evidence of selective action of paraphenylenediamine. It was in general agreement with the selective escape of certain dyes in the same region reported in a previous paper.[1] The thoracic and abdominal viscera showed no characteristic gross changes except for variable degrees of passive congestion. There was no excess free fluid present in the pleural and abdominal cavities. Careful necropsies in over 350 rabbits and cats failed to reveal edema of other regions of the body, including the subcutaneous tissues, except at the site of injection where there was a variable amount of edema due to local irritation from the compound itself, and from the acidity of the hydrochloride. Histologic examination of a rabbit that had marked edema of the head and neck showed no edema at the root of the tail. Accordingly, therefore, we were not able to confirm Gibbs's claim[2] that edema produced by paraphenylenediamine occurs also in the paws and at the root of the tail in both rabbits and cats. The results of our gross studies justify the conclusion that paraphenylenediamine produces edema of the head and neck regions only. This is sustained by our study of histologic changes associated with the process, now to be described.

Histologic Changes

Methods. The tissues studied histologically were obtained at necropsy from ten rabbits used in connection with another study,[4] the details of which will be reported later. All the rabbits received subcutaneous injections of 0.2 gm. of purified paraphenylenediamine hydrochloride per kilo, dissolved in about 10 c.c. of water. In addition to the paraphenylenediamine, six of the rabbits received other treatments. Rabbit 219 received 11.6 mg. per kilo of nicotine subcutaneously in seven divided doses during the three hours of the experiment, and Rabbit 262, an infiltration of the left Gasserian ganglion with nicotine. In Rabbit 233 the left trigeminal nerve was cut in the skull with a Magendie neurotome ten minutes before the administration of the paraphenylenediamine. In Rabbit 247, the sensory nerves of the tongue, and in Rabbits 245 and 246, those of the tongue and neck, were cut and allowed to degenerate for about two weeks before the paraphenylenediamine was injected. These various procedures have been found [4] not to influence the usual course of the edema process. In addition to these rabbits, the tissues of two untreated rabbits were studied as controls.

Blocks of tissue removed at necropsy were placed immediately into Orth's solution for fixation. These were later embedded in paraffin. The sections were stained throughout with both hematoxylin–Van Gieson and hematoxylin–eosin solutions. The histologic changes in important organs of all rabbits studied are summarized in the accompanying Table. Fig. 1 illustrates a section of the tongue of an untreated rabbit for comparison with that of a typical edematous tongue after paraphenylenediamine illustrated in Fig. 2. In order to give some quantitative idea of the edema in the various organs and in different animals the following designations and their signs were adopted: Slight (+), for just recognizable edema; moderate (++), marked (+++), and extreme, (++++).

Head and Neck. The presence of edema in these regions was readily recognized by the wide separation of the tissue cells and cell-groups from one another, and by the presence of fibrin in the spaces. The quantity of fibrin present was often marked. Fig. 2 illustrates the marked separation of muscle fibers frequently observed in the tongue. The results in the Table leave no doubt that the most pronounced edema occurred in the tongue, larynx and

TABLE

Degrees of Edema Following the Subcutaneous Injection of Paraphenylenediamine Hydrochloride in Rabbits (0.2 gm. per kilo) (1)

Number of rabbit	Tongue	Larynx	Neck tissues	Masseter muscle	Thymus gland	Sub-maxil-lary gland	Other tissues
261 (control; untreated)	–	–	–	–	–	–	
334 (control; untreated)	–	–	–	–	–	–	Pectoral and Thigh Muscles –
230	++++	++++	+++	+++		+	Pectoral and Thigh Muscles –
253	+++	++	++++	++	–	+	Pectoral Muscle –
219 (2)	+++	++++	++	+++	–	+++	Skin of Nose ++
262 (3)	++++	++++	++++	+++	–	–	Pectoral Muscle –
233 (4)	++++	++	+++	+++	–	–	Pectoral and Thigh Muscles –
247 (5)	+++	+++	+++	Hyoid Muscle ++		++	Thigh Muscle –
245 (6)	++++	+++	+	++	+++		Thigh Muscle –
246 (6)	+++	+++	+++	+++	++	+	

1. In the table the negative sign (–) means no edema, and the positive sign (+) means edema was present with different degrees rated as follows: slight (+), moderate (++), marked (+++), and extreme (++++).
2. Nicotine 11.6 mg. per kilo subcutaneously in seven divided doses in addition to the paraphenylenediamine.
3. Left Gasserian ganglion infiltrated with 0.25 c.c. of 1 per cent nicotine in addition to the paraphenylenediamine, subcutaneously.
4. Left trigeminal nerve cut ten minutes before paraphenylenediamine.
5. Sensory nerves of tongue cut, and degenerated two weeks before paraphenylenediamine.
6. Sensory nerves of tongue and neck cut, and degenerated two weeks before paraphenylenediamine.

loose tissues of the neck. The masseter muscle showed almost as much edema as these organs. The thymus and salivary glands occasionally showed moderate degrees of edema.

Discoloration of Tissues. More or less discoloration was observed microscopically in the tissues in which the edema was most marked. In some cases the mucous glands of the larynx appeared almost black. The muscle fibers of the larynx were often discolored, and commonly the masseter fibers were very dark. The tongue and the muscles of the neck were also variably discolored. The dark coloration of these areas suggests some chemical action between the paraphenylenediamine and the tissues affected, perhaps an oxidase reaction. The significance of it, if any, for the specific edema process has not been investigated, though it may be that it is fundamentally concerned with vascular injury in the edema regions.

Gasserian Ganglia. Histologic examination of the Gasserian ganglia of Rabbits 245, 246 and 247, whose sensory branches of the trigeminal nerves were cut and degenerated two weeks before the injection of paraphenylenediamine, revealed rather marked chromatolysis involving approximately from one-third to one-half of the cells. Allen [5] has described similar changes in the Gasserian ganglia of cats following section and degeneration of some of the sensory branches. The Gasserian ganglia of Rabbit 349 with the nerves intact, and receiving paraphenylenediamine, showed no changes. Hence, it appears that the changes in the Gasserian ganglia of the denervated rabbits were not due to the paraphenylenediamine. Sections of the cerebral cortex, cerebellum, medulla and lumbar cord appeared normal except for moderate congestion. These sections did not differ in appearance from those of an untreated control (Rabbit 352).

Viscera. In Rabbit 245 the heart muscle showed moderate edema. In Rabbits 219 and 230 there was moderate edema of the lungs, which was perhaps due to, or accompanied death from, circulatory and respiratory failure.

The *liver* in most of the rabbits showed evidence of abundant glycogen content. This was further demonstrated in two rabbits by a special stain (Best's carmine). In three rabbits the liver was the seat of a chronic inflammation, and to this group belonged one of the controls (Rabbit 261). Rabbit 247 showed marked proliferation of the bile ducts and surrounding connective tissue, probably

the result of infestation with *Coccidium oviforme*, although none of the parasites could be found in the sections.

The *kidneys* presented granular swelling of the tubular epithelium in four animals, one of these being a control (Rabbit 261). Two of these were rabbits in which there was chronic inflammation of the liver. The kidney changes in the three rabbits showing edema were probably not due to the paraphenylenediamine, since it had been shown previously by Tainter and Hanzlik [1] that renal functional efficiency during the edema was not impaired and the kidneys of their five rabbits showed no pathological changes.

The *pancreas* showed moderate edema in Rabbit 246 only. It was normal in all the other rabbits.

The *spleens* of all rabbits throughout were normal except for varying degrees of passive congestion.

Skin. We studied the changes in the skin after applications of 5 per cent aqueous and alcoholic solutions of the base and the hydrochloride daily for three weeks to areas of about 2 cm. in diameter of the shaved abdominal skin of a rabbit. The areas to which the base was applied appeared moderately thickened at necropsy, this change being greatest in the area receiving the alcoholic solution of the base, and was limited to the areas of application. However, no hyperemia or edema could be seen. The areas receiving the hydrochloride showed no differences from the untreated control areas.

Histologically, the treated areas of the skin showed extreme edema which was not confined to the dermis, but involved the underlying muscle to a moderate degree. The dermis was fully three times its normal thickness. The collagen fibrils in it were widely separated and a fairly large amount of fibrin was present in the spaces. The epidermis, which was very thin, appeared unchanged or normal. Discoloration and congestion were absent in the tissue sections. Further experiments were not made as the form in which paraphenylenediamine is present in various cosmetic preparations, etc., is not known or is uncertain. Therefore, evidence bearing on dermatitis from such preparations could not be satisfactorily studied. The presence of other constituents in these mixtures may easily modify the skin response, although the repeated application of free paraphenylenediamine base undoubtedly can cause considerable injury.

Conclusions

1. The systemic administration of paraphenylenediamine hydrochloride to rabbits and cats produced a marked edema in the tissues of the head and neck.

2. Gross and microscopic studies of various organs and tissues in rabbits showed that the edema process was not general, but was sharply localized in the head and neck regions, and, therefore, rather specific.

3. Histologically, the edema was characterized by a wide separation of the tissue elements and an extensive precipitation of fibrin from the exuded lymph. This ready coagulation of the fluid indicates a severe type of injury to the blood vessels, presumably in the capillaries.

4. This was further sustained by the presence of darkening (discoloration) in certain edema tissues from the poison, this phenomenon agreeing with the selective escape of dyes in these regions previously reported.

5. Repeated local applications of alcoholic and aqueous solutions of paraphenylenediamine base to the skin of a rabbit produced marked edema at the site of application.

We wish to express our thanks to Doctors Ophüls and Hanzlik for suggestions and criticisms of the manuscript.

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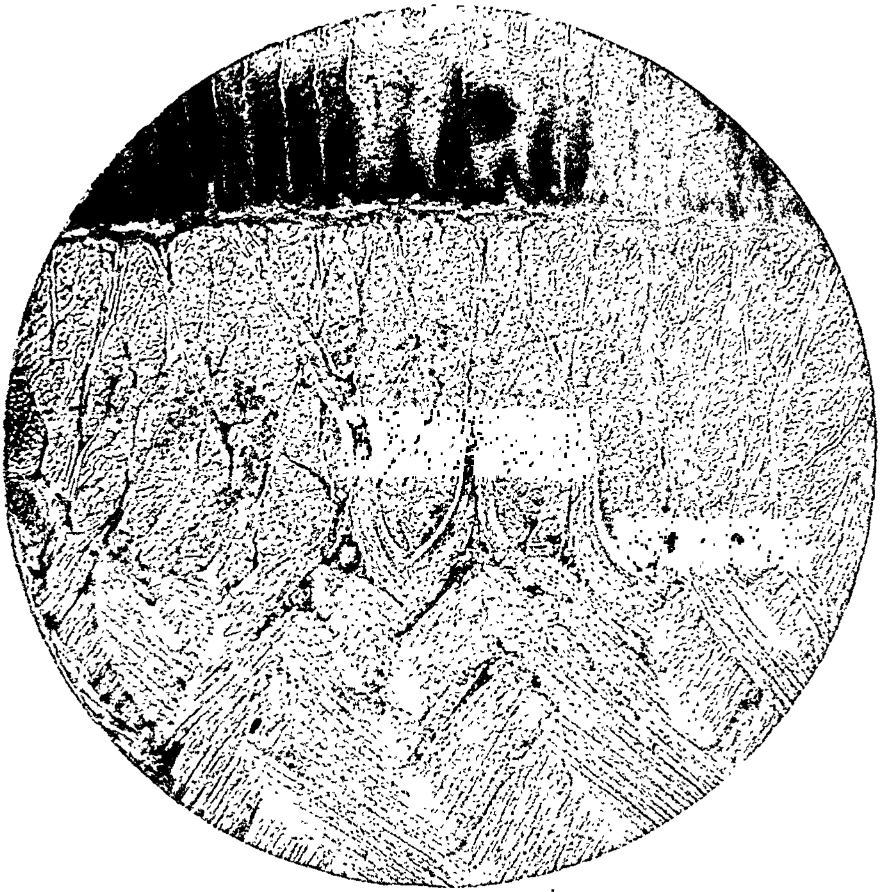
DESCRIPTION OF PLATE LXXXVI

Fig. 1. Section of tongue of untreated Rabbit 334 showing normal appearance of epithelium and muscle. (Low power.)

Fig. 2. Section of tongue (same region as in Fig. 1) of Rabbit 262 which received 0.2 gm. per kilo of paraphenylenediamine hydrochloride subcutaneously. Shows hydropic changes in the epithelium and extreme edema of the muscle with abundant fibrin. (Low power.)



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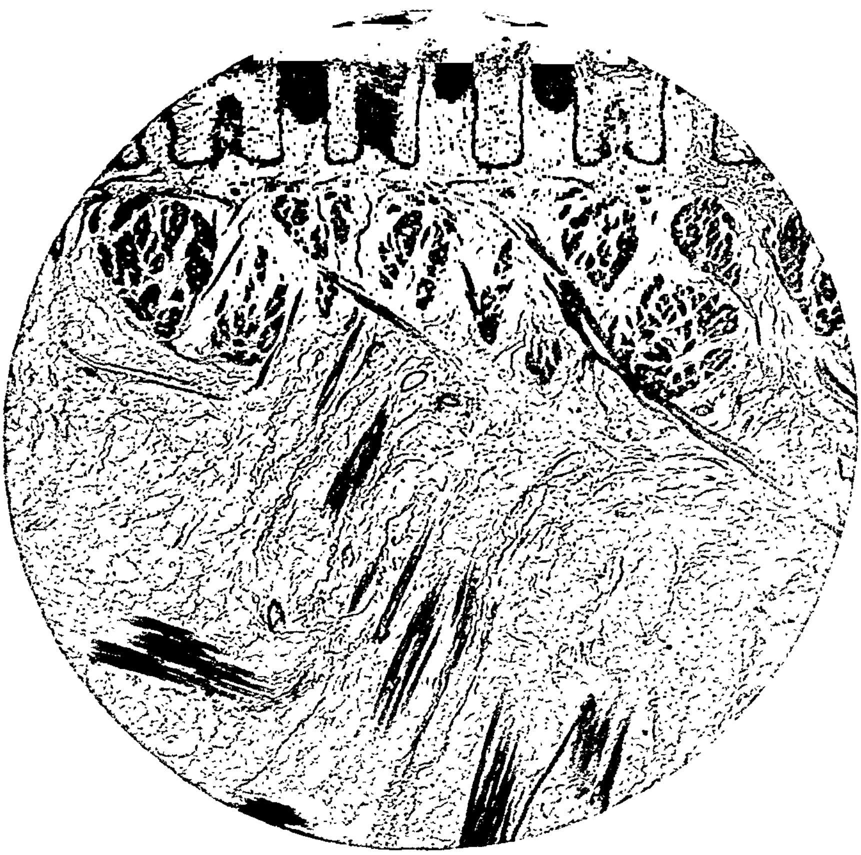

2

Tainter and Hall Edema of Paraphenylenediamine

MECHANICAL IRRITATION AS ETIOLOGIC FACTOR OF CANCER *

CLINICAL OBSERVATION

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Virchow's[1] "Irritation Theory," promulgated in 1855, has remained the most plausible and fruitful hypothesis for the etiology of neoplasms. This theory states briefly that: "Irritation is the essential factor of neoplastic tissue proliferation." His general principles concerning neoplasms, concisely formulated as "*omnis cellula e cellula*" and many other of his principles have stimulated the study of tumors by persistent empirical-clinical observations and were largely responsible for the numerous other theories explaining the origin of tumors (Cohnheim, Ribbert, etc.). These clinical and morphological studies, in turn, have led to extensive and systematic experimental work which in the hands of such investigators as Fibiger, Rhodenburg, Yamagiwa, Ichikawa, Bloch and others, of recent years, has brought out startling and convincing evidence in favor of the theory.

One of the earliest general principles laid down by Virchow declares that "For the production of vital activity of any part of the body, excitation or, in other words, irritation is necessary." Applying this principle to the formation of neoplasms, he made the deduction that "The production of large groups of cells from single ones occurs in the adult body unquestionably as the result of direct irritation of the tissues."

By means of analysis of a rich collection of empirical observations and experimental data, Virchow developed further details of the irritation theory. He recognized numerous single factors, as for example: "In case of mechanical irritation caused by a thread, the swelling is due to division of cells." "If the skin becomes irritated in consequence of continued friction and the irritation is increased to a certain point, the epithelium will thicken and, if the proliferation is very energetic, it may lead to tolerably large tumor-like

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formations." "The same effect, cell proliferation through cell division, may also be produced by chemical irritation as by the application of a caustic substance." As definite clinical examples of such irritations, Virchow referred to lip cancers of pipe smokers, and to cancer of chimney sweepers. It was through the study of the latter that the importance of tar as a chemical irritant has been demonstrated, and that the isolation of a chemical compound from tar by Bloch led to the production of cancer in animals almost at will.

Besides the action of chemical, mechanical and thermic irritants (*causa occasionalis*), emphasis is laid upon the importance of the local reaction of tissues with subsequent thorough and complex changes comparable to those occurring in the fertilization of the ovum after the action of the spermatocyte. This tissue reaction forms the second important factor in tumor formation, the *causa praedisponens*. In a long series of diverse conditions of local pre-disposition, Virchow emphasized the importance of *locus minoris resistantiae;* for instance, where derivatives of ectoderm and entoderm meet (orifices of the body, lip, anus, etc.), or in the viscera, the narrowed places (flexures, etc.) of the intestines which, because of their positions, are more exposed to irritation. The hereditary element of the local predisposition, is demonstrated in the congenital naevi (*n. verrucosus, n. pigmentosus,* etc.). On the other hand, as examples of acquired predisposition, Virchow [2] brings up a series of post-inflammatory changes: scars, polypi, etc., and emphasizes mainly the general fibrosis following chronic inflammation. The mucous membranes deserve mention particularly on account of the frequent hyperplasia, incited by the chronic inflammatory irritation, which in turn may often lead to real tumor formation (polypi).

The third important factor in production of neoplasms is the general *dyscrasia*, which however has to be strictly distinguished from *constitutional disposition*. This latter involves the changes of the tissue juices and blood as well as the alteration of all the body tissues proper. On the other hand, *dyscrasia* according to Virchow is always secondary to some local tissue changes. The essential changes of *old age* he found in various tissues. These are followed secondarily by *dyscrasia*. Various single factors usually combine in the production of tumors; for instance such is the case when an undescended testicle (developmental defect) is exposed to trauma, a

combination of local misplacement and mechanical irritation. The various irritants, as single factors, usually combine for the production of neoplasms; according to Virchow, "inflammation never admits of a single explanation; we can find side by side all the forms of irritation."

This tumor theory opened a wide experimental field for cancer research which however has only recently made rapid progress. For such work accurate clinical and morphological observations form a firm working basis. The purpose of this paper is to record a singularly interesting clinical observation in which the mechanical factor seems to be predominant in the production of cancer. The subsidiary factors must necessarily play a significant rôle, but the degree of their importance is obscure.

Case Report

Patient W. L. K., male, 60 years old, white, was admitted to the service of Dr. K. I. Sanes in the Western Pennsylvania Hospital, Pittsburgh, Pa., on Aug. 15, 1915, with the chief complaints of (1) abdominal pain, (2) constipation, (3) loss of weight, (4) anorexia.

Family History. Mother strikingly emaciated, died of old age.

Past History. Negative.

Present Illness. Began about one year before admission to the hospital with obstinate constipation. Six months later patient began to suffer from severe abdominal pain, which was pelvic in site and which often awakened the patient from his sleep. Later these attacks of pain with the same localization occurred during or immediately after meals. About two weeks before admission to the hospital patient had very severe attacks of griping pain of the same type as mentioned before, with nausea but without vomiting. His constipation became very bad and patient began gradually to develop obstructive symptoms. He lost 36 pounds in weight within the past six months (from 171 to 136 pounds).

Physical examination of the abdominal cavity revealed a moveable hard mass, probably of the sigmoid. Blood count was negative. Urine contained some acetone. Stool examination showed the presence of blood. Roentgenologic examination showed complete obstruction of the sigmoid somewhat below the end of the descending colon. Bismuth meal did not pass into the sigmoid. The bismuth column showed the same position at the end of thirty-eight hours. The bismuth enema passed this place although it did not fill up the descending colon.

Clinical Diagnosis. Carcinoma of the sigmoid.

Operation of sigmoidectomy was performed by Dr. K. I. Sanes at the Western Pennsylvania Hospital on August 19, 1915. A hard, constricting growth of the sigmoid was found below the junction with the descending colon. The growth had not invaded the serosa which had a smooth surface, but with some cicatrization of the meso-colon involving the inferior mesenteric artery. About 12 inches of the large intestine were resected with electric cautery. The growth occupied about the middle of the removal bowel. The operation was compli-

cated by interference with the blood supply of the lower bowel. The inferior mesenteric artery had to be ligated, as it was involved in the above-mentioned cicatrix. Consequently the descending colon was removed for the most part and the remnants of the colon and the sigmoid were united in an end to end anastomosis. The abdominal cavity showed nothing unusual otherwise. The liver was slightly enlarged, but no hard nodules were discernible in it.

The patient's convalescence was uninterrupted and after complete recovery he was discharged from the hospital on September 13, 1915. The last roentgenologic examination, in March, 1922, revealed the formation of a slight diverticulum at the place of the anastomosis. The patient, however, had made no complaints; his bowel movements and digestion have given him no trouble and he is still enjoying good health at present.

Pathologic Report

Gross Examination. Specimen consists of a piece of large intestine 30 cm. in length (Fig. 1). At about its middle portion a circular constriction is seen, which is hard in consistence and is covered with scar-like depressions and fibrous tags, but nothing else is noticed on the outside. Above the constriction the bowel is obviously distended, its wall is leathery and thickened as compared with the portion below the constriction. On opening, the knife meets with increased resistance when cutting through the constriction. The lumen shows a striking picture. There is an annular, ring-formed elevation constricting the lumen. It measures 4.5 cm. in width and averages 2.5 cm. in thickness. On cut surface a gray growth is seen with bacon-like, transparent appearance which shows gradual transition at its base to tendinous fibrous tissue, and at its periphery to yellow, subserous fatty, material. This ring-formed constriction has rather sharp, elevated edges and a depressed, crater-like middle portion. The surface of the crater is covered with hemorrhagic and necrotic material. The lumen here measures about 1.5 cm. in diameter and is filled completely by a round polyp. This has a mulberry surface covered with mucus and is soft in consistence. It forms the head of a long pedunculated growth with a broad attachment 8 cm. above. The stem is fan-shaped at its attachment (8 mm. at its base) and tapers to a neck where it measures 3 mm. in thickness. The round head of the polyp has a valve-like action and can be dislodged from below. It fits in snugly in the crater-like center of the annular growth, as a ball in a socket. On cross section, the head of the polyp shows soft consistence and rich mucoid secretion of the surface layer. A peach stone is lodged above the entrance of the constriction and

presses against the polyp. The stone is slightly larger than the lumen at the constriction and has otherwise the usual, rather rough surface and edges.

Above the constriction the mucosa is somewhat thickened and covered with mucus. The musculature shows obvious hypertrophy. The muscle layer measures 4 mm. in places. On one side there is a peculiar, rather superficial pouch lined with mucosa as elsewhere but with strongly developed musculature. The pouch seems to have been the nest for the peach stone.

Microscopic Examination. Section taken from the annular constriction at the crater-like ulceration shows a malignant epithelial growth. The glandular structure of the growth is well preserved in most places. The transition from the normal epithelial covering to the irregular epithelial proliferation can be followed distinctly (Fig. 2). The malignant cells vary a great deal in size and shape. In the glandular portions they are rather cylindrical with irregularly placed nuclei which show numerous mitotic figures. In the scirrhous portion, the cells are quite small. In the central portions the surface is necrotic. In the deeper layers the cells are smaller and surrounded by large amounts of dense fibrous tissue. This scirrhous growth, however, has not invaded the subserous tissues. The stroma increases in amount towards the deeper layers and contains a marked infiltration of lymphocytes. Section of the head of the polyp shows a benign epithelial tumor (Fig. 3). The surface shows typical intestinal glands, mostly perpendicular to the surface. In the deeper layers the glands run an irregular course and consequently they are cut in various planes. The cells are very regular in every respect. They are cylindrical with nuclei placed near the basal membrane. The blood vessels are well formed. Around the hilum they have also a muscular wall and are surrounded by fibrous stroma which contains scant lymphocytic infiltration. The surface epithelium consists mainly of goblet cells with increased mucus secretion which also covers the surface of the growth. Sections of the stem and base of the polyp show dense fibrosis and rich lymphocytic reaction. There are quite a few eosinophilic leukocytes present. Some sections of the intestinal wall above the constriction show marked hyperplasia of the smooth muscle cells. Van Gieson's and Mallory's connective tissue stains reveal an increase of fibrous tissue with diffuse lymphocytic infiltration.

Anatomic Diagnoses. Scirrhous carcinoma of large intestine; pedunculated adenoma of large intestine; dilatation of intestine and muscular hypertrophy of intestinal wall above constriction.

Discussion

From the pathologic study of the specimen it is evident that we are dealing with a carcinoma of the sigmoid, which originated where the head of the polyp caused friction of long duration. Obstruction was brought about by the ball valve-like mechanism of the polyp and cancerous constriction. Above this there is a marked muscular hypertrophy of the intestinal wall. Primarily this hypertrophy was due to the effort of the intestine to expel the polyp. This effort of expulsion pulled out the polyp and produced an unusually long stem (8 cm.). Unquestionably a long time was necessary to accomplish this. Later on, when a constriction was brought about and thus prevented the progress of the peach stone, the attempt to expel the stone produced an additional muscular hypertrophy by the same mechanism, with the result of a pouch-like irregularity of the intestinal wall. These conditions seem to indicate a well-marked mechanism of long duration. The formation of the polyp was probably due to a previous chronic inflammatory process. There is strong evidence for the causative relation between the head of the polyp and the cancerous growth, which corresponds accurately to each other as to location where the friction occurred, as a ball in a socket. This is particularly striking in view of the long stem. All of this seems to exclude a mere coincidence. The peristalsis of the intestines produced the continuous to-and-fro friction which had been increased by the effort of the intestines to expel the polyp and the peach stone. The head of the polyp, perhaps, did not remain in the place of constriction for a long period. This may explain the clinical observation that the patient never had complete obstruction. Still another factor of mechanical irritation may be added in the nature of fecal concretions which seem to play an important rôle in the production of cancer at the various flexures of the large intestine.

There are numerous empirical observations as to neoplasm caused by chronic mechanical irritation. Bashford[3] reported cases of melanosarcoma occurring in natives of Africa, which are caused by thorns causing continuous irritation of the sole of the foot. Cancers caused

by irritating foreign bodies (pessaries, etc.) have often been reported. Very striking also is the "cancer of the horn core" of Indian cattle, which develops at the root of the right horn used for attachment of agricultural implements. No cancer develops on the left horn of these cattle. Stones in gallbladder and renal pelvis as well as cancers of the tongue, which correspond to bad teeth and lip cancers of pipe smokers, have an inflammatory element in etiology besides the pure mechanical irritation. In the case reported in this paper, both the cancer and the polyp have the inflammatory factor in their etiology. This is unquestionably of primary importance particularly as far as the formation of the polyp is concerned. The proof of this is seen in diffuse fibrosis and lymphocytic infiltration at the base of the polyp. Post-inflammatory fibrosis has been recently emphasized in the so-called precancerous-lesions. The older and ancient authors used the term of "scirrhous induration" not only for cancers, but also for inflammatory fibrotic lesions. Galen[4] stated that *polypi aut inflammatione aut tuberculo. . . .* Galen emphasized the importance of inflammatory lesions and "other nodules" preceding the formation of cancers besides the general dyscrasia, *atra bilis.*

This has been given a strange, literal interpretation, whereas it represents the humoral pathologic conception of Hippocrates and Galen. It has been accepted also by Virchow, although he considered the humoral changes secondary to the tissue changes as seen in old age. Local cancerous predisposition of the sigmoid can be assumed on an embryological basis, also shown by the peculiar development of the mesosigmoid as contrasted with the mesocolon above and below it.

Conclusions

1. The case of carcinoma of intestine reported in this paper appears to present a striking example of mechanical irritation as an etiological factor in the histogenesis of cancer.

2. The case is analyzed and best understood by means of Virchow's "Irritation theory." According to this any of the single factors of three groups may be predominant in the histogenesis of cancer, which, however, is usually the result of various combinations of them.

These groups are, (*a*) *Causa occasionalis*, as the various irritants: mechanical, chemical, or thermic.

(*b*) *Causa predisponens*, as the embryological anlage or post-inflammatory changes.

(*c*) *Dyscrasia*, as general constitutional change.

The various well-known cancer theories and recent experimental works (Conheim's, Ribbert's tar cancers, etc.) represent only emphasis and elaboration of single factors in Virchow's comprehensive theory.

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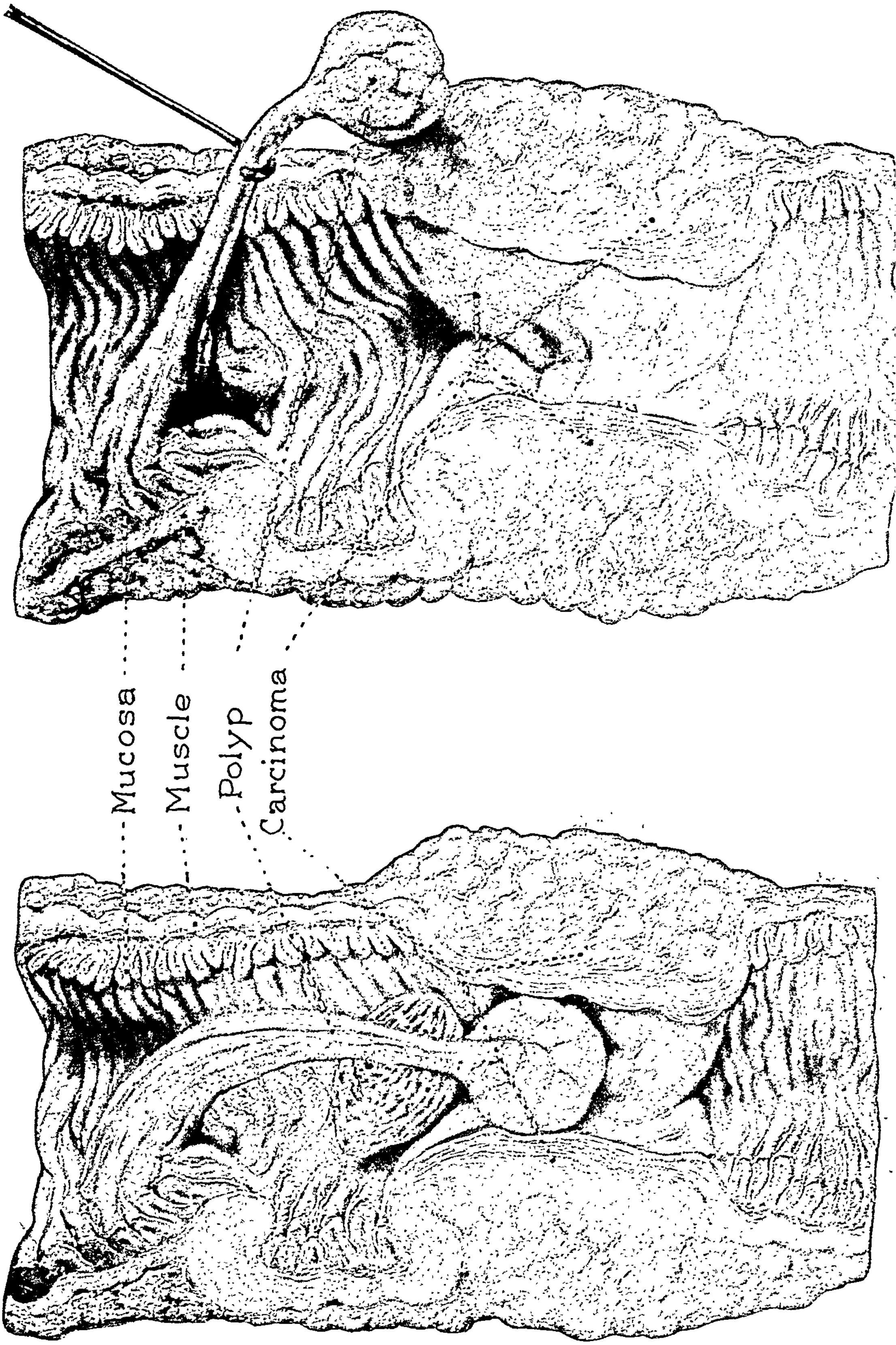
DESCRIPTION OF PLATES LXXXVII–LXXXVIII

Fig. 1. Drawing of specimen, showing lumen of intestine. *At left:* Annular constriction of scirrhous carcinoma. Lumen is completely plugged by head of polyp with long stem (ball in socket). Above head of polyp is the peach stone. *At right:* Polyp is lifted out of its nest. Peach stone is removed.

Fig. 2. Photomicrograph of section of intestinal wall, showing transition of normal epithelium to malignant proliferation. X 35.

Fig. 3. Photomicrograph of section of head of polyp, showing intestinal adenoma. X 35.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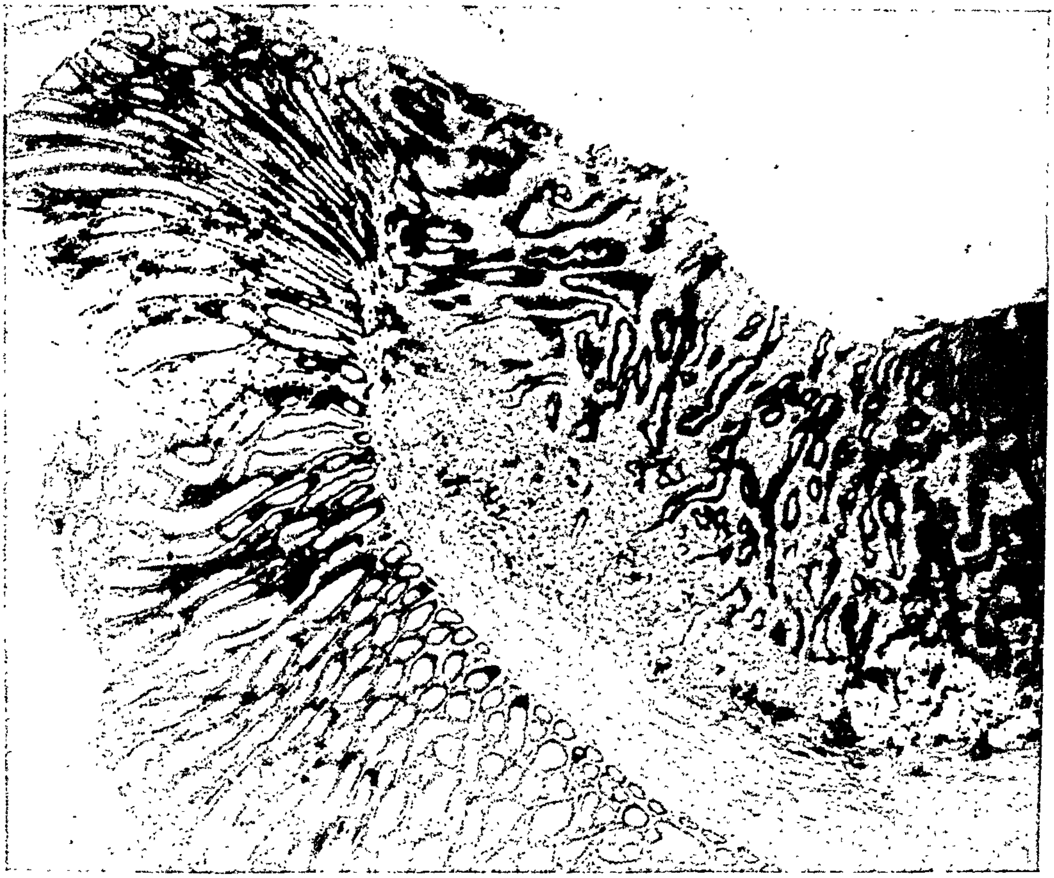

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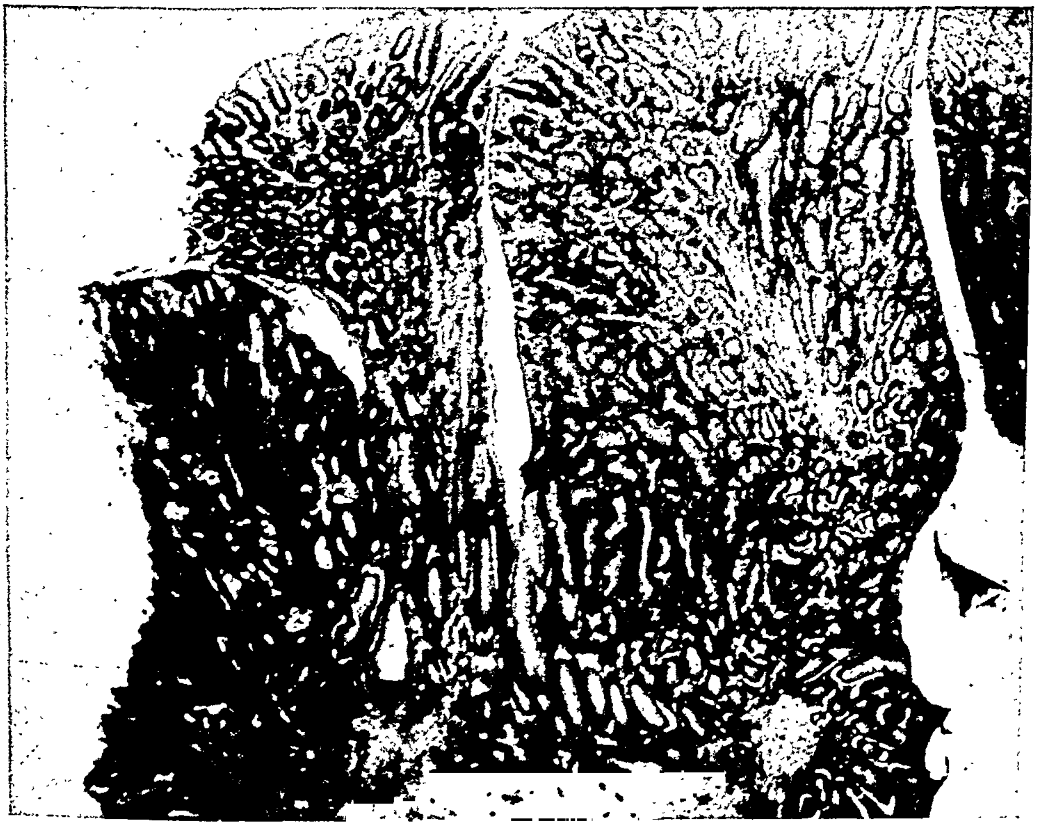

3

NEUROBLASTOMA OF THE INTESTINE *

Saul Alfred Ritter, Ph.D., M.D.

(From the Department of Pathology, Mount Sinai Hospital, New York City.)

Embryologists agree that the primitive undifferentiated nerve cells are of ectodermal origin, and early in the course of the histogenesis of the nervous system these cells differentiate into spongioblasts and neuroblasts. The former develop the supporting neuroglia structures of the central nervous system, while the latter are the precursors of neurons and also of the migratory cells which spring from the ganglionic crest, the anlage of the sympathetic nervous system. Some of these neuroblasts migrate ventrally toward the aorta to form the sympathetic chains with their paraganglionic structures and also invade the mesodermal part of the suprarenal glands to form their medullae. It is believed by some (Kohn [1]) that such primitive nerve cells may come to rest anywhere in the body, remain in a latent state and at some later time may proliferate and become neoplastic.

Marchand, in 1891,[2] reported a tumor of a suprarenal gland in a child 9 years old, which was rich in cells and fibrils, resembling the tumor now recognized as neuroblastoma. The presence of the fibrils led him to consider it a glioma.

Küster, in 1905,[3] reported two similar cases; one, a child 3 months old, presented a primary tumor of the right suprarenal gland with metastases in the liver; and a second case in which the tumor was also primary in the suprarenal gland, not complicated by metastases. He described undifferentiated nerve cells associated with fibrils, the latter staining brown with Van Gieson's method. He was the first to draw attention to the occurrence of a peculiar cell-grouping, the so-called "rosette" formations. He considered these tumors malignant gliomata.

Wiesel,[4] in the same year, called attention to the similarity between the cellular picture of the tumors described by Küster, and that of primitive sympathetic structures and of the medulla of the suprarenal gland.

* Received for publication July 6, 1925.

In 1910, J. G. Wright [5] reported four similar cases and definitely described the neoplasm as a neuroblastoma. He was able to demonstrate a very close similarity in its structure to the primitive sympathetic nervous system and the medulla of the suprarenal gland. This work was later confirmed by Landau.[6]

The latter reported three cases, and stressed the fact that the fibrils need not necessarily be present in these tumors. He believed that the more malignant the tumor, the fewer the fibrils and the more cellular its structure; and suggested that the younger the host, the more embryonic in character and more malignant is the tumor. He also showed that ganglioneuroma can be regarded as merely a more mature and more highly-differentiated form of neuroblastoma.

Pick and Bielschowsky [7] conversely pointed out that malignant neuroblastoma is the unripe counterpart of ganglioneuroma and therefore named these tumors *ganglioma embryonale sympatheticum.*

Pick, in 1912 [8] brought the number of cases up to eighteen, including one of his own. He discussed the histogenesis and relation of this form of tumor to the primitive nerve cells, and drew attention to the fact that in maturing the cells differentiate into higher, more specialized forms, the ganglion and paraganglion cells. Further contributions to the subject of neuroblastoma with extensive reviews have been made by Wahl,[9] Harbitz,[10] Lehman,[11] and R. A. Lambert.[12] Wolbach and Morse, in 1918,[13] brought the total number of undoubted cases up to twenty-nine, including three of their own. They, however, did not include the two cases published by Harbitz. Since then, only a few cases of this relatively rare neoplasm have been reported, Gunby,[17] Anderson and Shennan,[18] and J. H. Berner.[19]

The sites of primary occurrence in the reported cases were the suprarenal glands, sympathetic ganglia, retroperitoneal tissues, coccygeal gland, nose and uterus.

The observations to date have indicated that the term neuroblastoma must be reserved for tumors composed only of the undifferentiated type of nerve cell. On the other hand, tumors composed of ganglion cells with well-defined nerve fibers are called ganglioneuroma; and, since they are highly differentiated, are usually benign, in contradistinction to neuroblastoma. The third form of neurogenic tumor is the paraganglioma which is composed largely of chromaffine cells.[21]

Where mature ganglion cells have been found in typical neuro-

blastomata, they are to be regarded as forms in which partial transition has taken place from the primitive neuroblast to the mature ganglion cell. (Pick,[8] Landau,[6] Martius,[14] Wahl,[9] and Dunn.[15])

Martius reported a rather unusual case in which the tumor presented two almost separate parts, one simulating neuroblastoma, the other a ganglioneuroma. Other authors have reported cases of ganglioneuroma in which there occurred inclusions or islands of neuroblastoma (Pick und Bielschowsky,[7] Freund,[16] Dunn,[15] and others). Mixed neurogenic tumors containing all three elements of ganglioneuroma, neuroblastoma and chromaffine cells have been reported by Wahl [9] and others.

The two cases of neuroblastoma which I wish to add to the series already reported are unique because of their location and origin. In both instances the tumor arose in the jejunum, presumably from the nerve cells of the intestinal sympathetic plexus (Meissner's and Auerbach's). A search of the literature has failed to reveal reports of the occurrence of neuroblastoma arising in the intestine either primarily or by extension. The relative rarity of neuroblastoma occurring in the adult is an additional feature of interest.

Report of Cases

Case 1. Patient E. A., a widow, 55 years old, was admitted to Dr. Brill's service at Mount Sinai Hospital, New York City, on March 1, 1924, complaining of increasing constipation for four months. She attributed her complaint to an accident which occurred in 1923, when she was struck by a taxicab and sustained an injury to her right hip. The constipation was obstinate and required drastic cathartics for relief. There were no abdominal pains nor cramps, no nausea, vomiting, jaundice or melena. Her stools were usually firm, formed and dark brown in color. She had lost about 30 pounds in the preceding four months. Her past history was essentially negative. She had been a widow for twenty years and was never pregnant. There was no history of operations or serious illnesses. She had passed the climacterium twelve years ago.

Physical examination on admission to the hospital showed her to be well developed but markedly emaciated. There was moderate bilateral exopthalmos. The pupils reacted sluggishly to light. The heart was slightly enlarged and a systolic murmur was audible over the apex. The abdomen was distended. On palpation a large, oval, smooth, hard and immovable mass could be felt in the epigastrium, extending downward to the level of the umbilicus, more to the right than to the left of the midline. There was marked generalized voluntary rigidity of the abdominal muscles. The genitalia, aside from marked senile atrophic changes were negative. Examinations of the stools gave repeated positive guaiac reactions. The blood count, blood Wassermann test, and blood chemistry were negative. The phenolphthalein test revealed excretion of 80 per cent of the dye in the urine in two hours. Roentgen ray examination of the

colon with the aid of a barium enema showed the sigmoid to be markedly compressed by an extrinsic intra-abdominal mass.

An exploratory laparotomy by Dr. J. Brettauer disclosed a large neoplastic mass, densely adherent to the anterior abdominal wall. On separating the adhesions as well as the adherent omentum from the upper aspect of the tumor, the latter was brought into full view. The mass was about 8 cm. in diameter, grayish in color and had a rough surface. It appeared to occupy the upper portion of the mesentery and in it were incorporated loops of the upper portion of the jejunum. The large intestine encircled the mass, but was not invaded by it.

Five days after the exploratory laparotomy, the patient died from an extensive postoperative bronchopneumonia.

Necropsy. The body was that of an emaciated woman 55 years of age. Rigor mortis was complete. No icterus or petechiae were present. A 15 cm. median, recent abdominal operative incision was present which had been closed with interrupted sutures. Upon opening the peritoneal cavity a large, oval, firm, grayish-white, neoplastic mass measuring 15 x 10 cm. in diameter was found, which involved several loops of jejunum 5 cm. distal to the fossa duodenojejunalis and the contiguous mesentery. There were many recently formed fibrous adhesions between the parietal peritoneum of the anterior abdominal wall, omentum and the anterior surface of the neoplasm. The tumor apparently arose in the wall of the jejunum and by direct extension involved the walls of several adjacent loops of the jejunum and extended backward, distorting and invading the mesentery and the mesenteric lymph nodes in this region for a distance of about 5 cm. from its main attachment. As the result of erosion of the walls of the adjacent loops of intestine by the invading neoplasm, an intercommunication was established among several loops of the jejunum so as to form a cavity, the wall of which was formed partly by intestine and partly by tumor. The anterior surface of the mass, after separation of fibrino-purulent omental adhesions, was found to be greenish-yellow in color and necrotic in places. The free surface of the cystic cavity of the neoplasm was thrown up into large, firm, salmon-pink colored, irregular excrescences, covered by a film of thick mucus. Here and there necrotic areas were found. Cut section of solid portions of the tumor presented a homogenous, pinkish-white, friable surface.

The duodenum and the jejunum proximal to the site of the neoplasm were widely dilated, due to partial constriction of the lumen of the jejunum at the site of the neoplasm. The distal portion of the jejunum and the ileum were collapsed. The large intestine encircled

the neoplasm without being involved in the growth. On the basis of gross observation a tentative diagnosis of sarcoma of the intestine was made (Figs. 1 and 2).

The lungs were the seat of an extensive postoperative bronchopneumonia. The suprarenal glands were normal.

Examinations of all other organs revealed no significant findings.

Microscopic anatomy. Microscopic study of the tumor shows it to be essentially composed of numerous small, round, "lymphoid" cells, containing deeply stained nuclei, rich in chromatin granules and surrounded with a very little cytoplasm. Scattered throughout the microscopic field, a fine fibrillar stroma is seen which stains brown with Van Gieson's method but does not give the characteristic neuroglia stain with Mallory's hematoxylin phosphotungstic acid method. The cells present an alveolar arrangement and here and there are polarized in double rows about a central mass of finely granular material, assuming characteristic "rosette" formations. Some of the cells are somewhat larger in size and show mitotic figures. An occasional giant cell can also be seen. A few pyriform cells are present whose cytoplasm extends outward in the form of delicate fibrillar processes. No mature ganglion cells can be found. Numerous thin-walled blood vessels are seen in the reticular network supporting the cellular elements (Figs 3 and 4).

The microscopic characteristics of this tumor led us to group it with the undifferentiated type of neuroblastoma. This diagnosis was concurred in by Dr. F. S. Mandlebaum and by Prof. Ludwig Aschoff who examined the sections during his recent visit to New York City.

Case 2. Patient, J. F., white, male, Russian, 64 years old, was admitted to Mount Sinai Hospital on December 17, 1924, service of Dr. A. A. Berg. The patient had complained of severe epigastric pains and distress for several months with loss of weight and occasional vomiting. There was marked constipation. No hematemesis or melena had been observed.

On physical examination the patient was found to be markedly emaciated. His heart was slightly enlarged and the sounds were of poor quality. An indefinite resistance was felt in the left hypogastric region, but no distinct tumor mass could be palpated.

Roentgen ray examination of the gastrointestinal tract revealed a partial obstruction high up in the small intestine. The stomach was negative except for slight residue after six hours.

On December 12, 1924, an exploratory laparotomy was performed, under local anesthesia, by Dr. Berg. He found a neoplasm involving the jejunum commencing about 7.5 cm. from the fossa duodeno-jejunalis and extending dis-

tally in the wall of the intestine for a distance of 8 cm. There were metastases in the adjacent mesenteric lymph nodes. Many small metastatic nodules were present on the serous surface of the jejunum, just adjacent to the main tumor. Twenty-two centimeters of the jejunum were resected together with a portion of the mesentery, containing tumor and enlarged glands. An end to end anastomosis was then made in the usual manner. The omentum was sutured over the anastomosis with interrupted catgut sutures and the abdomen closed without drainage.

The patient made an uneventful recovery. His appetite increased and his bowels moved without catharsis. His general condition improved very rapidly and he was discharged from the hospital on July 18, 1924.

The macroscopic specimen consisted of a portion of jejunum which measured 22 cm. in length and 9 cm. in its widest diameter, the narrowest diameter being 4 cm. Attached to the serosal surface infiltrating the mesentery and wall of the intestine there was a cellular growth measuring 5 x 2.5 cm. This neoplasm was situated directly opposite the narrowest portion of the jejunum, namely the area which measured 4 cm. in diameter. It involved the wall of the intestine at this point and extended distally for a distance of 8 cm. The mucosa of the involved portion of intestine was irregular, nodular and firm. On the serosal surface there were several small, round or oval, firm, neoplastic elevations which on section were similar to the main neoplasm (Figs. 5 and 6).

Upon microscopic examination the tumor is found to be made up of small, round "lymphoid" cells whose nuclei stain deeply and contain chromatin granules. The stroma of the tumor is composed of a fine, irregular, diffuse, fibrillar network which does not give the characteristic neuroglia stain. Scattered throughout the microscopic field are many pyriform-shaped cells with a round base, and pointed apex which gives off a fine fibrillar process. Their deeply staining nuclei are oval in shape, vesicular in character and contain many chromatin granules. Many giant cells of variable sizes are also present, some of which show mitotic figures. They are not of the foreign body variety (Fig. 7).

An interesting point in the histological structure is its resemblance to spongioblastoma, a form of neoplasm derived from the primitive glial elements of the central nervous system described by Strauss and Globus.[20]

Summary

Two cases of intestinal neoplasma are reported. They are grouped with the neuroblastomata because of their histological characteristics.

In both instances the tumors arose in persons past middle age. Metastases were found only in the adjacent mesenteric lymph nodes. The relatively benign character, at least in one of the tumors (Case 2), may, perhaps, be explained by its histologic resemblance to a form of tumor designated as spongioblastoma.[20, 21]

The observations indicate a new and hitherto undescribed site for primary neuroblastomata. So far as can be ascertained, no similar cases have been reported in the literature.

These cases emphasize the necessity for careful histologic study of atypical tumors, which on first examination might appear to be sarcomatous in character. It is probable that for this reason other instances of neuroblastoma of the intestine have been classified as sarcomata.

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DESCRIPTION OF PLATES LXXXIX–XCI

Fig. 1. (Case 1.) Anterior view of intestinal tumor showing its origin and relations.

Fig. 2. (Case 1.) Undersurface of tumor showing also partial obstruction of the intestine.

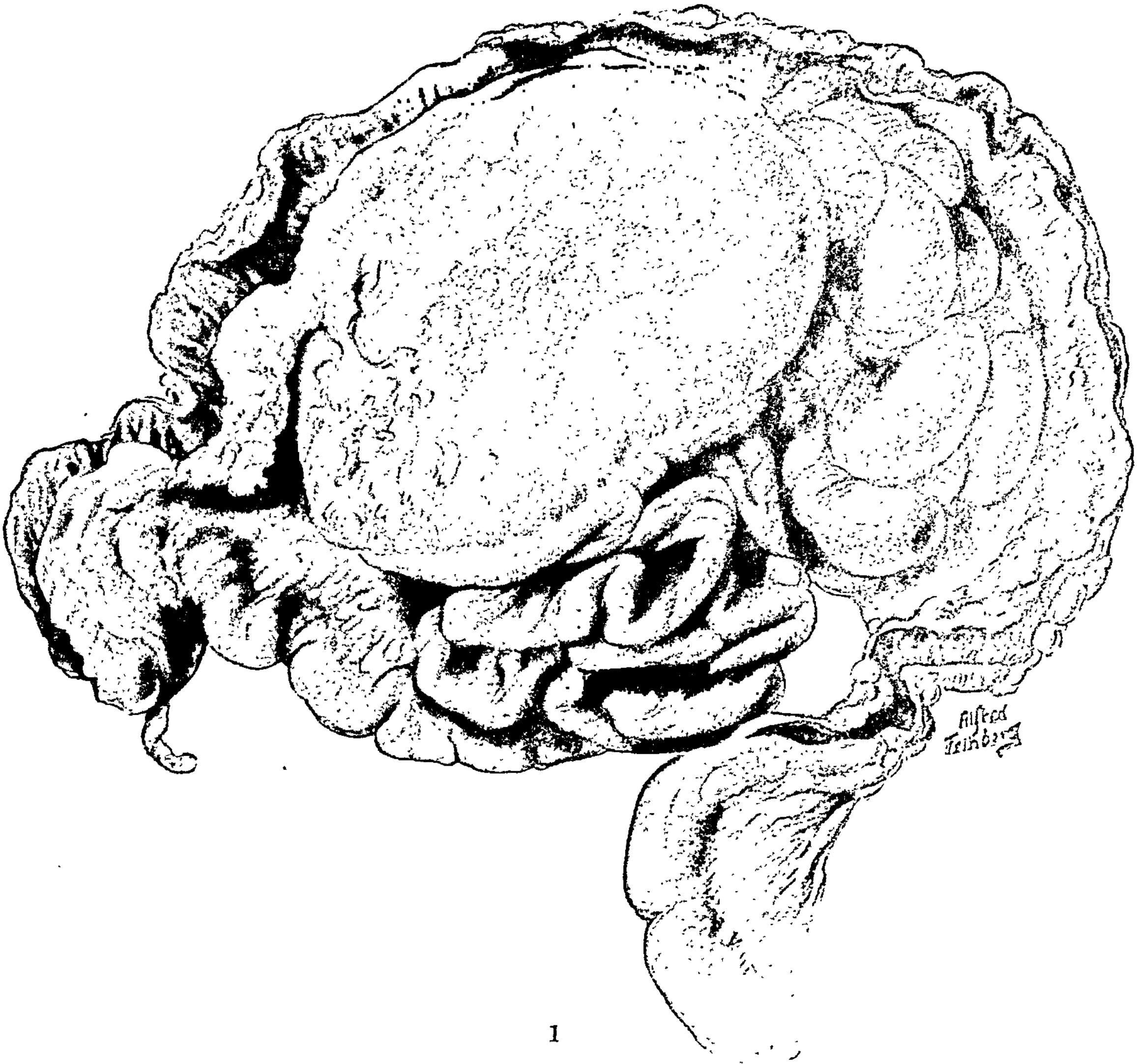
Fig. 3. (Case 1.) Photomicrograph of tumor showing "lymphoid" cells containing deeply stained nuclei surrounded by very little cytoplasm. Cells present an alveolar arrangement. A fine fibrillar stroma is scattered throughout the field. (High power.)

Fig. 4. (Case 1.) Section showing typical rosette arrangement of cells. (Higher magnification.)

Fig. 5. (Case 2.) Drawing of specimen of tumor of jejunum; view of mucosal surface.

Fig. 6. (Case 2.) Serous surface of tumor of jejunum with adjacent portion of invaded mesentery and lymph nodes.

Fig. 7. (Case 2.) Photomicrograph of section of tumor showing presence of large numbers of giant cells and numerous pyriform-shaped cells alternating with small "lymphoid" cells. For detailed description see text.



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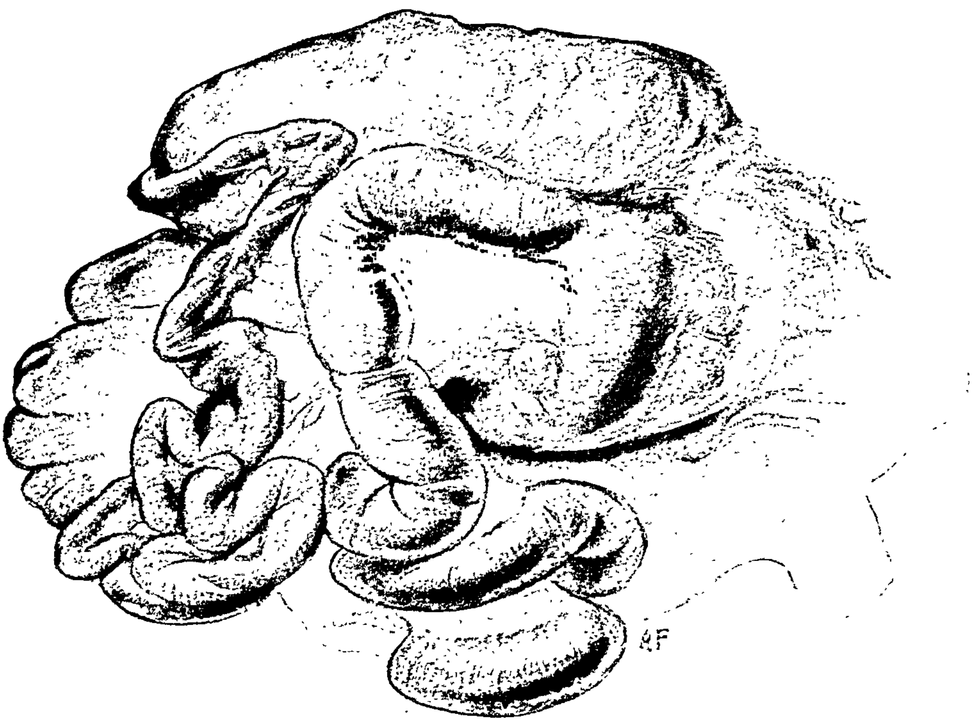

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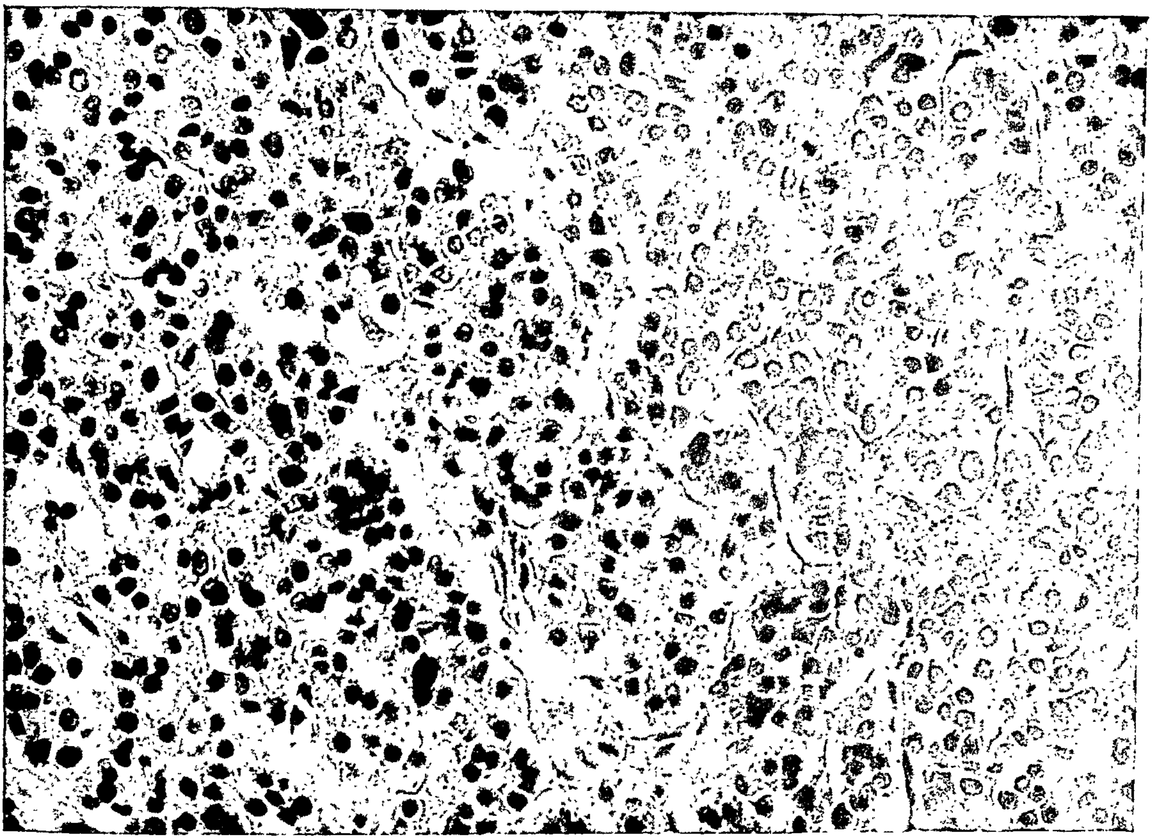

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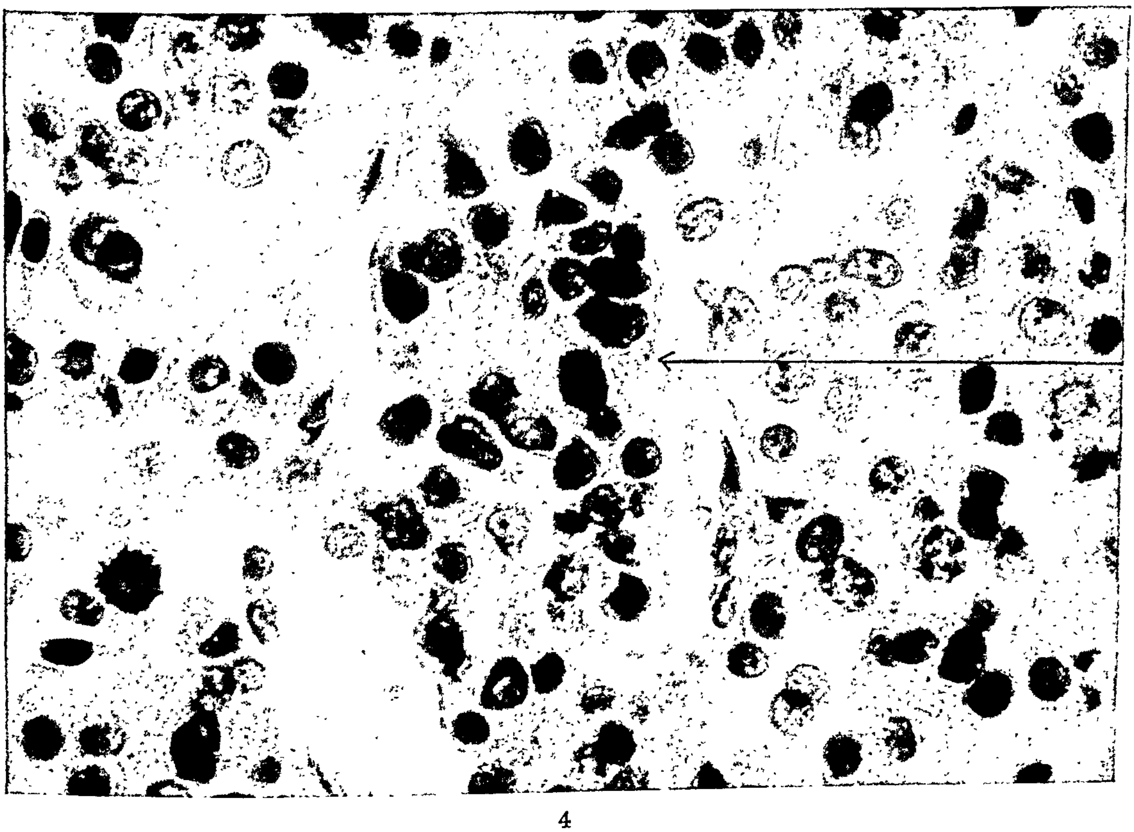

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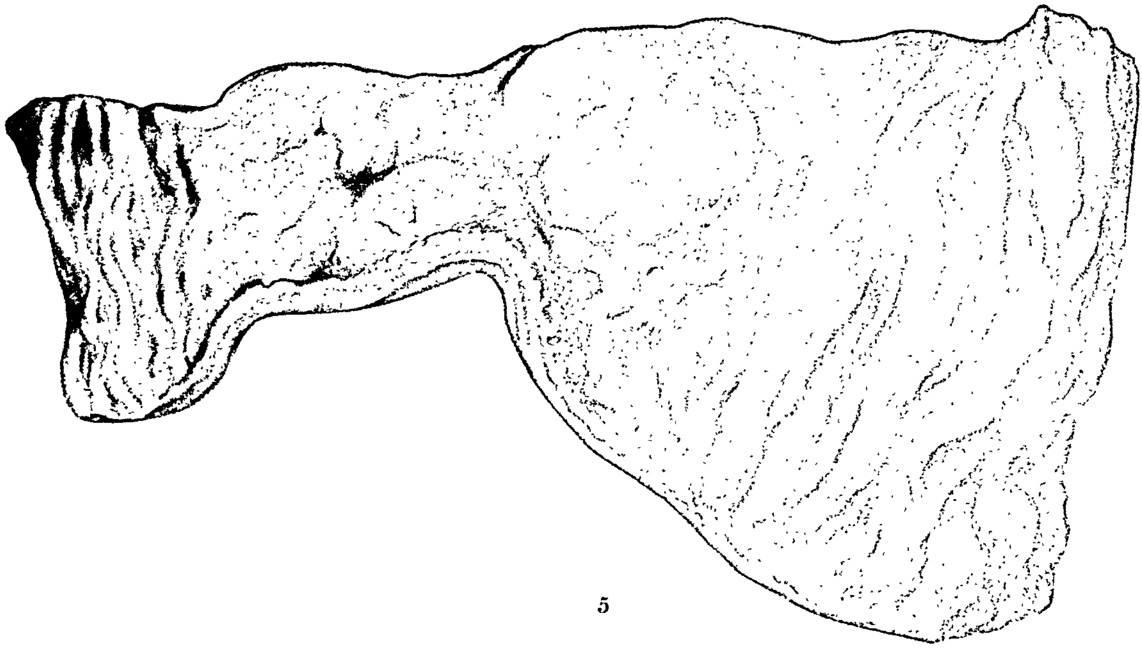

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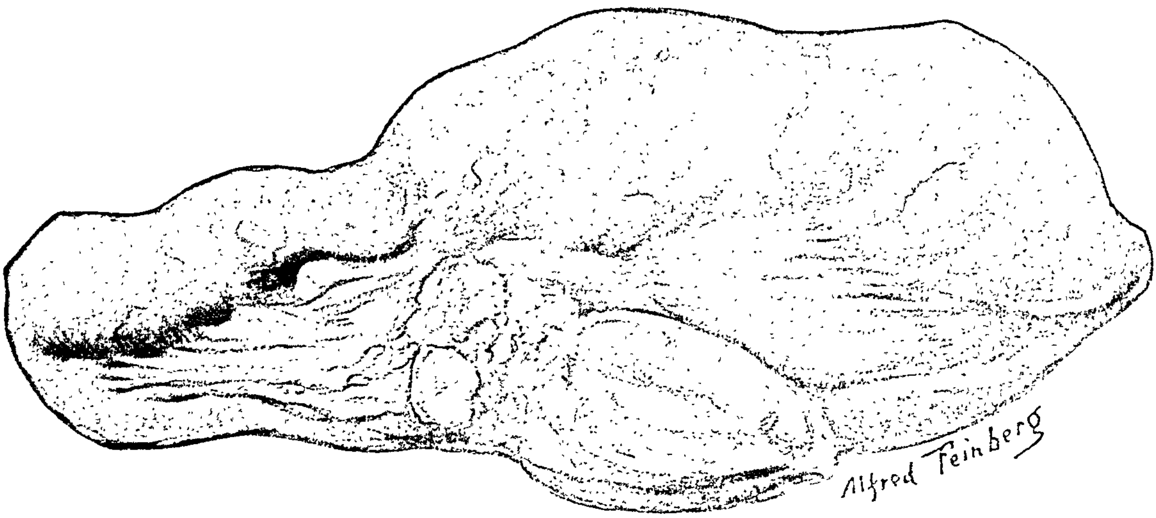

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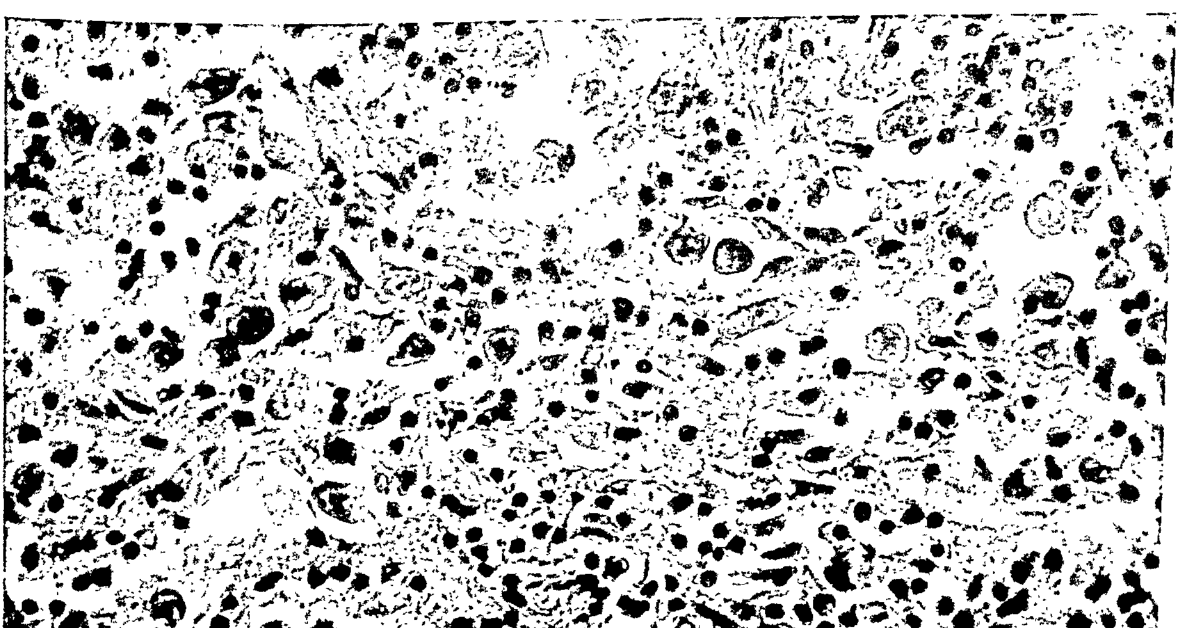

7

SCIENTIFIC PROCEEDINGS OF THE
TWENTY-FIFTH ANNUAL MEETING

OF THE

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

WASHINGTON, D. C.
MAY 5 AND 6, 1925

AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

SCIENTIFIC PROCEEDINGS

Owing to unavoidable circumstances it has been impossible to include the discussions.

THE VERNES FLOCCULATION TEST AS A GUIDE TO TREATMENT OF SYPHILIS.* A. B. Baylis, A. E. Sheplar, and W. J. MacNeal, New York.

Abstract. With adequate equipment the Vernes test on blood serum is less time-consuming than the Wassermann test and its results are recorded in precise figures to the second decimal place as milligrams of flocculation per cubic centimeter, through a range from −0.10 to +2.50. The Vernes reading has been aptly compared to the reading of a thermometer. It shows very slight fluctuations in health and in diseases other than syphilis. Marked fluctuations are observed in this latter disease, correlated with the activity of the pathological process.

In cases under treatment the Vernes test offers an additional criterion of considerable value in estimating the progress of the condition. If the reading falls consistently one feels confidence in his therapy. Conversely a persistent high Vernes reading suggests a revision of the therapeutic regime. The test represents a distinct addition to the serological control of syphilis.

DIPHTHERIA IMMUNITY. THE EFFECT OF REPEATED INJECTIONS OF AVIRULENT DIPHTHERIA BACILLI, B. HOFMANNI AND B. XEROSIS IN GUINEA-PIGS.* M. J. Rosenau and (by invitation) G. H. Bailey, Boston.

Abstract. The object of these experiments was to determine whether avirulent diphtheria bacilli are able to stimulate antitoxin production and thus account for the acquired immunity to diphtheria which most persons develop with maturity. Seventeen different strains of avirulent cultures, and also cultures of related organisms such as *B. hofmanni* and *B. xerosis*, were injected in guinea-pigs over a long period of time. All of these guinea-pigs remained Schick-positive and when finally tested showed no immunity to diphtheria toxin. It was found that there is considerable individual variation in guinea-pigs so far as their response to injection of diphtheria toxin is concerned. Usually, guinea-pigs become Schick-negative and immune in three months. One of our animals remained Schick-positive after thirty-nine injections of small doses of diphtheria toxin (1/125 to 1/8 M.L.D.) over a period of fifteen months. This guinea-pig received a total of 1.94 M.L.D. If experiments such as ours, with avirulent cultures, could be continued over a series of years, it is possible that some immunizing effects might be manifest.

Our studies emphasize the essential difference between the so-called avirulent diphtheria bacilli and the true Klebs-Loeffler organism. Not only did the aviru-

* To be published in Archives of Dermatology and Syphilology.

* To be published in the Journal of Infectious Diseases.

lent strains fail to induce an immunity, but their agglutination reactions showed that there is a wide immunological gap separating them from the diphtheria bacillus. Some of these so-called avirulent strains have pathogenic properties. We found that a few of our guinea-pigs receiving repeated massive injections of live cultures developed disseminated focal lesions which are being studied.

No change in virulence was observed in cultures of avirulent diphtheria bacilli or *B. hofmanni* or *B. xerosis* under observation for eighteen months, and transferred for thirty-six generations on Loeffler's blood serum.

Guinea-pigs react with striking uniformity to the Schick test. All guinea-pigs appear to be like some children, in that they are persistently positive. The guinea-pig's skin seems to be more sensitive than the human skin, for a typical reaction is produced by the intradermal injection of 1/250 M.L.D. Pregnancy and general systemic infections appear to inhibit the local skin reaction.

Pseudo-reactions were not observed in our studies as a result of the injection of diphtheria toxin. But sensitized guinea-pigs responded to the injection of bacterial antigens — although this reaction is not specific. With certain bacterial extracts containing bacterial protein, a local necrotic action of the skin was produced which was interpreted as a modified Arthus' phenomenon.

Experimental confirmation was obtained of the correlation between a positive Schick test and susceptibility and a negative Schick test and immunity. In a series of guinea-pigs it was furthermore found that a definite relation exists between the weight of the animal and the M.L.D. of diphtheria toxin. It takes about twice as much diphtheria toxin to kill a guinea-pig weighing four times as much as the standard control. In other words, if the M.L.D. for a 250 gm. guinea-pig is 0.011 then it will take 0.22 for a guinea-pig weighing 1000 gm.

Morphologically the avirulent diphtheria bacillus resembles the true Klebs-Loeffler organism, but the results recorded above emphasize the fundamental difference between the two groups and throw doubt upon the appropriateness of the name "avirulent diphtheria bacilli."

Further Results with the Dick Test and Active Immunization with Scarlatinal Toxin. Abraham Zingher, New York.

Abstract. 1. The scarlatinal toxin can be standardized with only a fair degree of accuracy. Toxin prepared without blood should preferably be used to avoid confusing serum reactions.

2. Scarlet fever developed only in those who give a strongly positive Dick reaction — no case developed in a negative or slightly positive reactor.

3. The Dick test made on over 600 patients with scarlet fever showed that over 95 per cent had a slight or moderately positive reaction during the first few days of the disease and a negative reaction in convalescence. A persistent strong positive reaction in convalescence points against the diagnosis of scarlet fever. Patients admitted to the hospital as scarlet fever cases and showing such reactions should be immunized with a prophylactic dose of scarlatinal antitoxin.

4. The negative Dick reaction in the "naturally" immune is not as permanent as the negative Schick reaction; — about 10 per cent of children under 15 years of age gave a slight or moderately positive reaction when retested within a year. Among adults, however, only 1 per cent changed from a negative to a slightly positive reaction.

5. The dose of toxin for immunization has been increased, four injections, 250, 1000, 2000, 3000 skin test doses, being given at weekly intervals.

6. The rapidity in the development of immunity after scarlatinal toxin injections indicates that immunization with toxin can be used during the outbreak of scarlet fever. Care must be taken, however, under these conditions not to mistake constitutional reactions associated with rashes as clinical cases of scarlet fever. Such reactions develop in 5–10 per cent of individuals after the first dose of toxin and in only very few after the subsequent injections.

7. The antitoxic immunity after scarlatinal toxin injections develops much sooner than after diphtheria toxin-antitoxin injections. Of those who gave a negative Dick reaction at the end of one month, 20 per cent showed slight or moderately positive reactions at the end of eight to twelve months, but none had so intense a reaction as in the original test. With a second series of toxin injections complete immunity developed in these individuals and also in those who failed to be fully protected after the first series. With a sufficient number of doses of the toxin a large proportion of human beings can be successfully immunized and kept immune against the toxic effects of scarlet fever.

8. The ultimate duration of the immunity conferred by injections of scarlatinal toxin cannot be predicted at the present time. The wide distribution of the scarlatinal streptococcus makes it appear very probable that the immunity process, once successfully started, will continue in most of the persons injected as a result of contact exposure and repeated infection with the specific organism.

Rapid Alterations in the Blood Sugar Level of Rabbits in Anaphylaxis and Following Injections of Bacterial Proteins. Isolde T. Zeckwer (by invitation), Boston:

Abstract. In fatal bacterial anaphylaxis produced in rabbits, there were found to be rapid alterations in the blood sugar level. When determinations were made on blood withdrawn by heart puncture at short intervals, there occurred, after latent period, a gradually ascending curve of blood sugar, when symptoms of anaphylaxis were beginning to be manifested; then usually a relative decrease; followed by an abrupt rise to an extremely high level at the time of death, with values varying from 321 mgm. per 100 c.c. blood to 1060 mgm.

In order to determine whether this effect was due to the anaphylactic state or to the effect of the introduction of killed bacteria into the blood stream, suspensions of killed organisms of various types were injected intravenously into unsensitized rabbits and blood sugar determinations made at short intervals. In the cases of *B. coli* and *B. proteus* isolated from normal human feces, and with a stock culture of *B. paratyphosus B*, there was a gradual increase in the blood sugar level, reaching a maximum in about ninety minutes, and gradually returning to the original values in six to eight hours. The maximum values reached were 321 mgm. with *B. proteus*, 244 mgm. with *B. coli* and 208 mgm. with *B. paratyphosus B*. With *B. enteritidis* of Gaertner there was only a slight increase to 132 mgm. The increase in blood sugar with the two latter organisms is apparently in accord with the findings of Menten and Manning, who reported before this Association last year the effect on the blood sugar, of organisms of the paratyphoid B. enteritidis groups recovered from animals suffering from spontaneous infections by these organisms. Bacteria which produced no noteworthy effect upon the blood sugar level were *B. typhosus*, *B. paratyphosus A*, *B. fecalis alkaligenes*, *B. pyocyaneus*, staphylococci, hemolytic streptococci, and non-hemolytic streptococci.

The character of the curve of blood sugar in anaphylaxis and the maximum height attained were quite similar whether anaphylaxis was produced by the

injection of organisms which in themselves altered the blood sugar level in the unsensitized animal, or whether by those organisms which had no such effect. In fact, the highest value of blood sugar in anaphylaxis was produced by the injection of killed non-hemolytic streptococci, which had repeatedly been shown to have no effect on the blood sugar in the unsensitized animal.

In considering the mechanism of the production of these blood sugar changes, the phenomena cannot be due to failure in the utilization of glucose. The extreme heights of the blood sugar level attained in such short periods of time can only be accounted for by increased production of glucose through rapid mobilization of glycogen. The effect is apparently similar to the increased glycogenolysis produced by adrenalin and by stimulation of the sympathetic nerves to the liver.

Bilharzia in the Appendix. Alfred Plaut, New York.

Abstract. The purpose of presenting this paper is to emphasize three facts: (1) the occurrence of Bilharziosis in the large cities of the East, (2) the possibility of complete absence of symptoms from intestine, (3) the fact that the position of the spine in the egg is not characteristic for a different species of bilharzia.

The patient F.T., colored, 26 years, living in New York for many years, was in the hospital with the diagnosis: Retroversion, Endocervicitis. Her complaint was profuse discharge and occasional backache. Operation was done for retroversion, the lacerated cervix was amputated; the appendix gave the impression of subacute involvement and was taken out. Nothing in the previous history or in the findings pointed toward an intestinal condition.

The gross appearance of the appendix was normal, length 7 cm., thickness normal, serosa slightly injected. The microscope shows many tubercle-like formations in all layers of the wall. They consist chiefly of swollen connective tissue cells and of some eosinophiles. In their centers the eggs of *Distoma* (*Schistosoma*) *haematobium* are found. Many of the eggs are very well preserved, in one of them a typical larval stage (Miracidium) is found, the contents of most of them indicate that they contain young developing embryos. The spines are easy to be seen in many instances, their characteristic outline can be distinguished without difficulty from shrinking and other artifacts. Some of the spines are polar, others are lateral. Eggs with polar spine and eggs with lateral spine are found nearly in the same microscopic field. Some of the destroyed eggs apparently are situated in small hyalinized blood vessels; they may have been caught there with their sharp spine. Some tissue reaction is present around all eggs found in the appendix. All transitions are found from intact eggs to complete destruction. The débris of the shell is partly surrounded by foreign body giant cells, while otherwise very few giant cells are found. Delicate onion-shell-like structures mark the place where the destruction of an ovum has been completed. The shell stains distinctly with Weigert's elastic stain. The situation of the eggs bears no relation to the direction of muscle fibers.

In the cervix, which showed papillary erosion, one calcified egg was found and one of the above mentioned onion-shell-like structures. The cervix was dissolved in antiformin but no further eggs were found.

The feces contained few eggs of bilharzia, the urine none. There was no eosinophilia in the blood. The Wassermann was positive.

The patient made an uneventful recovery and left the hospital. Her infection must date far back as the calcified egg shows. How far her abdominal organs

are the seat of bilharzia remains an open question. Laparotomy has been followed by unexpected improvement even in very severe cases of bilharziosis. *Trichocephalus dispar* was present in the feces but not in the appendix. A similar case was published from this hospital a few years ago.

PLEXIFORM NEUROMA OF THE PHARYNGEAL MUCOUS MEMBRANE. Louise H. Meeker, New York.

Abstract. A neuroma involving a whole plexus of nerves, that is, a plexiform neuroma, may occur as one of the manifestations of a general v. Recklinghausen's neurofibromatosis, or it may occur alone. In the present case a plexiform neuroma involved a large part of the plexus of the glosso-pharyngeal nerve on the right side of the throat. The patient was a girl, 14 years of age. A swelling back of the posterior pillar appeared as a fairly hard tumor-like mass, 6 cm. in diameter. There was no ulceration. Extirpation of the growth in the pharynx was somewhat difficult and nine irregular pieces of tissue were removed.

Plexiform neuroma in the pharynx has not been previously reported. In the gross specimen the translucent, vermiform nerve trunks could easily be teased out by aid of the dissecting microscope.

The striking thing in every section was the abundant convoluted nerve trunks, larger and smaller, ramifying throughout a scanty connective tissue stroma in every direction. Mucous glands and muscle bundles were invaded by the tortuous nerves.

The nerves show well-preserved medullated nerve fibers and abundant ganglion cells in clumps or embedded singly among the fibers.

Many ganglion cells have a capsule as in the sympathetic system. Certain ganglion cells and nerve fibers appear to be related to each other. The tissue immediately about the nerve fibers and ganglion cells is neurogenous tissue.

There has been much discussion as to the exact nature of the growths. They have been compared to harmatoma (Albrecht), have been considered dystrophies (Herxheimer and Roth) in contradistinction to neurofibroma (v. Recklinghausen), or true neuroma (Durante, Verocay, Askanazy, Wallner). All agree to an underlying embryonal malformation but as Herxheimer and Roth remark, "there is no agreement as to where malformation stops and tumor begins."

The plexiform neuroma in this case seems to stand in an intermediate position between the plexiform neuromas of Verocay and Herxheimer and Roth on the one hand and the ganglion neuromatosis of Krauss or Obendorfer on the other hand.

CONGENITAL SARCOMA OF THE LEG (Bone Sarcoma?). REPORT OF A CASE WITH EIGHT YEARS' CURE. Anatole Kolodny, Iowa City.

Abstract. An infant girl eight weeks old with a congenital tumor of the left leg, a Roentgenogram showing bone involvement suspicious of bone sarcoma was admitted to the Iowa State University Hospital in 1917. An amputation through the middle of the thigh followed with a permanent cure as regards a recurrence to date.

Pathology. A tumor which replaced practically all of the soft tissues of the leg, covered with normal skin, proved to be a sarcoma with a perivascular arrangement in the younger portions and a seeming differentiation into a

fibrosarcoma in the older. The relation of the tumor to the bones of the leg could not be decided from the morphology of the tumor. The very intimate relation of the tumor to the bones, especially the fibula, with the evident involvement of the bones on Roentgenogram would seem to speak for bone origin of the tumor.

Renal Neoplasms in Young Children. Martha Wollstein, New York.

Abstract. Seventeen primary tumors of the kidney were studied grossly and microscopically. Six occurred in infants under one year of age, nine in children between one and three years, and two in patients three and a half and six years old respectively. All were unilateral, retroperitoneal, within the kidney capsule and compressed the kidney substance so that a layer of fibrous tissue from which all renal structures had been eliminated separated neoplasm and kidney. Any portion of the kidney may be involved. Seven were firm in consistence. Ten were soft, even fluctuating in places.

Microscopically all the growths were embryonal in type. There was one spindle cell sarcoma and two adenomata. In the other fourteen tumors embryonal epithelial elements and embryonal connective tissue elements varied in comparative quantity and arrangement, defining several sub-groups.

The less differentiated the component cells, the more rapid, soft, and invasive the growth, and the more fatal the prognosis. The more highly differentiated the cells, the firmer and less invasive is the growth, and the greater is the possibility of cure.

The more solid tumors are more easily removable, because they remain within their capsule unbroken. From the three recovered cases they were all removed entire. The more rapidly growing tumors break through their capsule and recur locally; they may also metastasize in the lungs and liver. Metastases occur through the blood stream.

Unusual Modes of Dissemination of Ovarian Carcinoma.* Isaac Levin and (by invitation) Joseph Barnet, New York.

Abstract. In the course of the last year the writers have observed amongst the pathological material at the New York City Cancer Institute a series of cases of ovarian carcinoma in which the secondary tumors have invaded the vital organs and caused clinical symptoms stimulating primary conditions in such organs.

In ovarian carcinoma the secondary tumors developed most frequently not through multiplication of cancer cells which were transported as an embolus through the lymph or blood circulation but by direct implantation of the cancer cells on the parietal or visceral peritoneum, or by Handley's method of lymphatic permeation.

The cases reported in this presentation showed invasion of the stomach, the body of the uterus, and the cervical endometrium. Clinically the cases simulated primary carcinoma of the stomach and carcinoma of the body of the uterus and the cervix. A detailed report of the clinical history and postmortem findings is given.

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THE EFFECT OF THE SCORBUTIC STATE UPON THE PRODUCTION AND MAINTENANCE OF INTERCELLULAR SUBSTANCES. S. B. Wolbach and (by invitation) Percy Howe, Boston.

Abstract. This paper deals with the effort to determine the fundamental lesion or defect of the scorbutic state and the endeavor to make a pathological characterization of scorbutus. So far, guinea-pigs in the condition of absolute scorbutus, *i.e.*, complete deprivation of anti-scorbutic substances in the diet, have been followed, and the effects of the administration of anti-scorbutics noted. The effects have been studied in growing guinea-pigs and in the repair of lesions of bone experimentally made. The earliest effect of the scorbutic state is to be found in the incisor teeth, and is evidenced by the manner in which the formation of dentine is affected and in changes in the layer of odontoblasts. The earliest changes may be observed in six to seven days, while a few days later very striking conditions are found, among the most noticeable of which is the separation of the layer of odontoblasts from the dentine. This space is presumably filled by liquid. The odontoblasts undergo striking changes in regard to size, arrangement and staining reaction. The effect of the administration of an anti-scorbutic upon this condition is apparent within forty-eight hours and results in the prompt formation of dentine so as to fill the space caused by the separation of the layer of odontoblasts. In the bones, formation of bone ceases immediately, while osteoblasts in certain locations, particularly beneath the periosteum, continue to proliferate. This applies both to flat bones and long bones. Accumulations of osteoblasts of considerable size may occur before hemorrhages take place. That the cells under consideration are osteoblasts is proved by the effect of the administration of a single dose of an anti-scorbutic substance, because it is followed by the prompt appearance of bone matrix between the cells. A single administration of orange juice produces an effect which is easily demonstrable forty-eight hours later. In the incisions through the cortex of bone in the scorbutic state there is no bony repair, while controls operated on in the scorbutic state, but given anti-scorbutic substances after the operation, show new bone formation very promptly. The repair of soft tissues is likewise very markedly affected. Proliferation of fibroblasts is apparently but little affected by the scorbutic state, but there is a marked diminution in the amount of intercellular substance formed. There is also a very marked effect in the retardation of new blood vessel formation in the organization of lesions, etc.

Other observations were made upon the effect of the scorbutic state upon bone previously formed and upon cartilage.

The conclusions reached include the following, that the scorbutic state may be characterized as one affecting supporting tissues in which the cells are unable to produce and maintain intercellular substances. This condition affects various supporting substances to a different degree and is most marked in those in which the intercellular substance is calcified, as the dentine of teeth and the matrix of bone. The characterization applies to cartilage and connective tissue, and, by inference, to other intercellular substances, including that of blood vessels. The hypothesis is entertained, based upon the study of repair in incisor teeth of scorbutic guinea-pigs, that in the formation of intercellular substances there is a change of the material from a liquid to a solid or jell state, and that the missing factor in the scorbutic condition is one affecting the jelling or setting of a liquid product.

THE EPITHELIAL TISSUES IN EXPERIMENTAL XEROPHTHALMIA. S. B. Wolbach, and (by invitation) Percy Howe, Boston.

Abstract. This paper contains the results of the study of xerophthalmia in rats in the effort to characterize the condition pathologically. The conclusion is reached that the condition is one primarily affecting epithelia. While transitional stages may be observed, the condition of xerophthalmia results in the transformation of various epithelia into a stratified squamous keratinizing epithelium. This change has been observed in the upper respiratory tract, including the whole of the nasal passages, larynx, trachea, bronchi. In the digestive tract, the changes are most marked in the salivary glands and accessory salivary glands, and here cavities several millimeters in diameter may occur as a result of the retention of desquamated keratinized epithelial cells. Change has also been observed in the pancreas. No changes have been detected as yet in the epithelium lining the stomach and intestines. In the genito-urinary tract this transformation into keratinized epithelium is found in the renal pelvis, bladder, seminal vesicles and epididymis.

In all instances, in glands the earliest changes are to be noted in the ducts, and the histologic sequences which will be described later seem to be the same in all locations, indicating that a common factor is operative. Other changes observed include a striking atrophy of certain epithelial structures, notably thyroid gland and testes. Less striking atrophies occur in the paraocular glands and the pancreas.

The following conclusion is made: Deprivation of the anti-xerophthalmic (fat soluble A) vitamine effects specifically epithelial tissues. This effect is manifested in cells having presumably widely different chemical (secretory) functions and terminates in complete loss of specific function and in the transformation into a common type of chemically inactive (non-secretory) epithelium.

EPITHELIAL CELLS IN CONJUNCTIVAL INFECTIONS. S. Hanford McKee, Montreal.

Abstract. At the end of the third or fourth day of a gonorrhoeal ophthalmia, the clinical signs are well established. Chemosis is marked, discharge is profuse. Examination of the pus at this time may show a few gonococci but more likely no micro-organisms will be found. If now a smear be made by taking some of the epithelial cells and staining with Giemsa they will be found filled with gonococci. To the different forms of resistance met with by the gonococci as they are attempting to penetrate the epithelial cover we must add phagocytosis by the epithelial cells.

COMPENSATORY HYPERTROPHY OF THE THYMUS GLAND. J. Marmorston-Gottesman and (by invitation) H. L. Jaffe, New York.

Abstract. In the course of a series of experiments which involved the carrying out of over 100 thymectomies in rats at the age of puberty or younger, it was frequently noted that, when a small fragment of the gland was accidentally left behind at operation, autopsy disclosed a fairly large regenerated mass even as early as two weeks after the partial thymectomy. These incidental findings indicated that the gland was capable of undergoing rapid compensatory hypertrophy. But this evidence was indirect as we had no means of accurately estimating the amount of thymus left behind, and therefore a series of experiments was planned to obtain direct proof of compensatory hypertrophy.

A small number of young rats were partially thymectomized, the right lobe being completely removed; and an equal number of litter mates of corresponding sex were kept as controls. The environmental and nutritional conditions were the same for all animals. The rats were sacrificed twenty-one to twenty-four days after the beginning of this experiment; the thymus glands were removed and dissected from the surrounding fat and lymph nodes, and the individual lobes were immediately weighed. A clean dissection of the thymus from the fat and lymph nodes is a simple task in young rats.

The left lobes of the partially thymectomized animals averaged in weight 42 per cent above the left lobes of their controls; while the average difference in weight between the right and left lobes of the controls was only 5 per cent. It is significant also that the difference in body weights between the controls and operated rats was 3 per cent in favor of the controls.

These results clearly indicate that the thymus gland is capable of undergoing very rapid compensatory hypertrophy in young animals. Since hypertrophy is a response to increased physiological demands it would seem, in view of these experiments, that the organ has an important function particularly before puberty. These experiments offer further proof in support of the belief that the thymus is a gland of internal secretions.

EXPERIMENTAL ENDOMETRIOSIS. V. C. Jacobson, Albany.

Abstract. In a series of nineteen rabbits the intraperitoneal autotransplantation of endometrium during oestrus was successful in sixteen, or 84 per cent. In six rabbits so treated during the resting stage there was one positive result. In six rabbits operated on during pregnancy there were implantations in two, or 33 per cent. An increased vitality or "virulence" may be assumed for endometrial tissue during oestrus. This is in accord with Sampson's menstrual theory of origin of most cases of human endometriosis. Implantation usually occurs upon pelvic structures, and in a few instances it has been observed upon the colon and urinary bladder. The implants are invariably cystic adenomatoid structures, often multilocular. There is no evidence in these experiments in favor of the view that endometrial tissue can be formed by metaplasia of mesothelium.

CHEMICAL STUDIES ON POLYCHROME METHYLENE BLUE.* W. J. MacNeal and (by invitation) J. A. Killian, New York.

Abstract. Polychrome methylene blue is a mixture of various nearly related substances, among which methylene blue (tetra-methyl-thionin), methylene azure B (tri-methyl-thionin), methylene azure A (asymmetric di-methyl-thionin) and methylene violet of Bernthsen have been definitely identified. As a preliminary to further study of the application of these dyes in pathology the methods of preparing the dyes themselves have required attention.

Methylene blue. The medicinal methylene blue of commerce, while by no means a chemically pure substance, is satisfactory for staining work. Special brands, such as Ehrlich's rectified methylene blue, contain other dyes of this group, especially the azures. Methylene blue may be purified by recrystallization from hydrochloric acid, 20 per cent.

Methylene azure B. This substance, tri-methyl-thionin, was first recognized by Kehrmann in 1906, but the practical manufacture has been baffling. Our pres-

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ent method is as follows: Dissolve 16 gm. medicinal methylene blue in 4000 c.c. water, warm to 60° C. and add 150 c.c. of 10 per cent potassium di-chromate. Heat to 75° C., mix thoroughly and let stand in room overnight. Filter with suction. The slightly moist precipitate is transferred to a liter flask and mixed with 800 c.c. water, 5 c.c. of reagent formalin and 21 c.c. of concentrated hydrochloric acid (Sp. G. 1.19). A long reflux condenser is attached to prevent access of air and the mixture is boiled for four hours and transformed into a deep blue solution with the evolution of much formaldehyde. After very slight cooling add slowly through the condenser tube 25 c.c. of 20 per cent ammonium carbonate. Abundant carbon dioxide is evolved and the excess hydrochloric acid is neutralized. The condenser is then plugged and the mixture cooled in running water. When cold, add 350 gm. of pure sodium chloride, mix thoroughly and let stand overnight in a cool place. The precipitate is separated on suction filter, dried and recrystallized from ethyl alcohol. It finally consists of long slender needles and filaments, extremely soluble in water, from which it cannot be precipitated by hydrochloric acid. Upon addition of sodium hydroxide it becomes purplish-red and now the color may be almost completely extracted by ether, chloroform or ethylene dichloride.

Methylene azure A. Asymmetric di-methyl-thionin is made by dissolving 16 gm. medicinal methylene blue in 4000 c.c. water, adding potassium dichromate, 98 c.c. of 10 per cent solution, and 60 c.c. of concentrated hydrochloric acid (Sp. G. 1.19), and boiling for three hours. Protection from the air is unnecessary. Add 1500 gm. of pure sodium chloride, mix and let stand overnight. Recrystallize the precipitate from alcohol and dry it at 100° C.

Methylene violet. Methylene violet is the residue after removing a dimethylamine group from the methylene blue molecule and substituting an atom of oxygen for it. The reaction requires, first, oxidation and, second, hydration, in very dilute solution. Dissolve methylene blue, 8 gm. in water 5000 c.c. Add 49 c.c. of 10 per cent yellow potassium chromate and 10 c.c. of 10 per cent ammonium hydroxide. Boil for two hours. Add 15 c.c. of 30 per cent sodium carbonate and boil four hours longer. Cool and separate the precipitate on suction filter. Dry it at 37° C. and recrystallize from ethylene dichloride.

All these substances have thus been prepared in crystalline form with only moderate amounts of impurity. Their application in pathology may now be studied with greater promise of success.

THE PATHOGENESIS OF BILIARY CALCULI. S. H. Mentzer (by invitation), Rochester, Minn.

Abstract. There are two sites for the formation of biliary calculi: within the intrahepatic ducts, and in the lumen of the gallbladder. Intrahepatic stones are essentially inflammatory in origin and contain little or no cholesterin. Stones arising within the gallbladder probably can be aseptically formed and always contain varying proportions of cholesterol. Increase of cholesterol concentration of gallbladder bile may occur when the wall of the gallbladder is not capable of properly handling the cholesterol contained within it. Stasis and infection are not necessary for stone formation, but both are usually present. A nucleus of some sort is necessary for gallstone formation. A change in the acidity of gallbladder bile probably produces a precipitation of the material for stone formation.

THE PATHOLOGICAL EFFECTS OF SOLUBLE TOXIC SUBSTANCES OBTAINED FROM *B. Paratyphosus B.* Maud L. Menten, Pittsburgh.

Abstract. Soluble toxic substances were obtained from two strains of *B. paratyphosus B* (human — Brown) and PT2 isolated from rabbit and *B. enteritidis* by filtration of five-day broth cultures through Berkefeld filters and by various extractions and precipitations of suspensions of agar growths. All toxic substances recovered when injected intravenously into rabbits caused immediate rise in blood sugar reaching a maximum in two and a half to three hours and returning to normal in four to five hours. Paratyphoid toxin from animal strain gave the most marked hyperglycemia and this was followed by an eight- or ten-day interim of normal blood sugar values with a rise to about 200 mgm. per c.c. of blood, twenty-four or forty-eight hours before death.

Enteritidis toxin gave the least hyperglycemia on injection and these animals died with paralysis of hind limbs. All animals showed cellular degenerations in liver, spleen, adrenals, pancreas and kidneys. Spleen and liver were pigmented and infiltrated with laked erythrocytes. The most characteristic lesion of the paratyphoid toxin was focal necrosis of liver; chromatolysis of ganglion cells and round cell perivascular infiltration of the brain were constant when enteritidis toxin was used.

EXTRACTS OF NORMAL TISSUES IN EXPERIMENTAL TUBERCULOSIS.* Richard S. Austin, Cincinnati.

Abstract. The inoculation of saline extracts of certain organs of normal rabbits appears to influence the development of experimental tuberculosis in rabbits. The extracts of different kinds of organs vary considerably as to the amount of influence they appear to exert. Extracts of adrenal and lung would seem frequently to retard the development of lesions, those of heart and liver produce less effect, while extracts of spleen and kidney usually exert little or no influence. The freshness of the organs used, the short time utilized in preparing the extracts, and the preservation of the extracts at icebox temperature suggest the possibility that the more or less protective substances present in these organ extracts are of "native" occurrence, existing in the organs before removal from the normal animal.

LOCALIZATION OF STREPTOCOCCI FROM CASES OF EPIDEMIC NAUSEA AND VOMITING AND OF EPIDEMIC NEURO-MYELO-ENCEPHALITIS. E. C. Rosenow, Rochester, Minn.

Abstract. During the autumn and early winter of 1924, there occurred in various parts of the United States and Canada, in conjunction with relatively mild infections of the respiratory tract, an unprecedented number of cases of persistent hiccup, and cases of nausea and vomiting with or without hiccup, and of neuritis associated with or without myelitis and encephalitis. In certain communities hiccup was the most common symptom, in others nausea and vomiting, or deep-seated, unproductive coughing, while in still others neuritic pains, often unilateral, of scalp or forehead, or outspoken neuritis, dominated the picture. Marked changes in the character of the symptoms sometimes occurred during the course of the epidemic. This was true of the epidemic in Rochester. The cases of hiccup occurred chiefly between November 17 and

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December 9, 1924, and the cases of nausea and vomiting and of neuritic pains during December and the first three weeks in January.

The methods of study were similar to those used in a study of the etiology of epidemic hiccup and encephalitis. With a few exceptions, the findings in the animals paralleled in important respects those in the patients studied. The dogs injected with the material from the two patients, who were severely nauseated and vomited, developed anorexia and vomited repeatedly.

Evidence of neuritis was present in all of the five cases studied, and correspondingly, hemorrhagic lesions of nerves occurred in eight of twenty-one rabbits injected intracerebrally, and in ten of seventeen rabbits injected intravenously. Parallelism between findings in patients and animals is further shown by the fact that, of sixteen rabbits injected intracerebrally with the streptococcus from the three cases in which there was no evidence of myelitis, only one (6 per cent) had gross hemorrhages of the cord or medulla, while of twenty-two injected with material from the two patients who had undoubted symptoms of myelitis, nine (43 per cent) had gross hemorrhages in the cord.

The character of the microscopic changes in the animals injected in this series of cases was similar to that found in the experiments in epidemic hiccup and encephalitis. Hemorrhage, edema and leucocytic infiltration dominated the picture in the experiments of short duration, while later infiltration by round cells became predominant. The demonstration of organisms in the acute lesions was relatively easy; in the chronic lesions it was difficult and sometimes impossible. The streptococcus isolated in these cases is morphologically and culturally indistinguishable from the one I have isolated in epidemic hiccup and encephalitis. Like the latter, it is of relatively low general virulence. It is a slightly elongated diplococcus occurring singly and in short chains. It is gram-positive, non-encapsulated, bile-insoluble, and on blood agar produces small, dry, slightly elevated, non-adherent colonies surrounded by a green halo. All of the five strains were agglutinated by my polio-encephalitis immune serums, in dilutions as high as 1:100, and not by other similarly prepared immune serums. Positive precipitin reactions were obtained in the polio-encephalitis serums with the cleared suspensions of nasopharyngeal swabbings in three or four cases. Since changes in localizing power were noted in some of these strains following artificial cultivation and animal passage, the conclusion seems warranted that the changes in the character of the epidemic, from hiccup to other manifestations of a neuro-myelo-encephalitis, were due to change in the tropism or localizing power of the streptococcus isolated.

Experimental Glomerulonephritis in Monkeys.* E. T. Bell and B. J. Clawson, Minneapolis.

Abstract. Intravenous injections in rabbits of several strains of streptococci from human sources produced in several instances nephritis of the spontaneous type (lymphocytic interstitial nephritis) but did not result in glomerulonephritis.

Similar injections in monkeys resulted in two cases of severe nephrosis, one of acute interstitial nephritis, one of glomerulonephritis, and one of a type not yet determined, since the animal is still living.

The case of glomerulonephritis was characterized by epithelial crescents, fusion of glomerular lobules to the capsule of Bowman, swelling of the endothe-

* To be published in the American Journal of Pathology.

lium with occlusion of the capillaries, and atrophy of tubules associated with occluded glomeruli.

Glomerulonephritis has been produced in one monkey by intravenous injections of streptococci.

EXPERIMENTAL INTRACAPILLARY GLOMERULONEPHRITIS. F. B. Mallory and Frederic Parker, Jr., Boston.

Abstract. The injection of 0.5 to 1 gm. of metallic zinc in fine powder form subcutaneously into rabbits produced acute intracapillary glomerulonephritis in four out of eleven animals killed at times varying from three to seven weeks. The time required depends apparently on the amount of local inflammatory reaction. The invading leucocytes dissolve the zinc and set it free to be absorbed into the circulation.

Proliferation of the endothelial cells lining the capillaries in the glomeruli is often active, mitotic figures are fairly numerous (occasionally two in one section of a glomerulus) and the vessels are quickly distended and occluded by the newly formed cells. The glomeruli are enlarged, containing on section two to three times as many nuclei as a normal tuft, fill the capsular space and often project into the beginning of the tubule.

The glomerular lesions are often accompanied by more or less degeneration of the renal epithelium (hyaline droplet formation, necrosis, sometimes calcification) and by regeneration of renal epithelium. The tubules contain casts and the urine albumin. Injection of zinc salts caused marked local reaction and acute tubular nephritis in a few days. Evidently slow absorption over a considerable period of time is required in order to produce the glomerular type of lesion. Two of the rabbits developed paralysis of the forelegs.

The chief source of danger to man of poisoning with zinc is from inhalation of fumes and dust in occupations involving this metal. "The chronic interstitial nephritis and paralysis from which spelter workers suffer" is interpreted by T. M. Leggs as "sequelae of plumbism" due to the frequent presence of lead in zinc. The lesions described above throw considerable doubt on this statement. Other sources of danger are foods containing acids cooked in galvanized pails, and the light fluffy stearate of zinc used as a dusting powder for babies.

Metallic nickel in powder form causes a more intense local reaction than zinc, when injected subcutaneously into rabbits, but produces somewhat more slowly the same type of proliferative changes in the vessels of the glomeruli, namely, mitoses and distension and occlusion of the capillary vessels by the newly formed endothelial cells.

THE ACTION OF GERMANIUM DIOXIDE ON THE RABBIT. C. H. Bunting, Madison, and (by invitation) G. H. Bailey and P. B. Davidson, Boston.

Abstract. Germanium dioxide in doses as small as 4 mg. per kilo has not only failed to act as a hemopoietic stimulus in the rabbit but has proved to be toxic to heart, liver and kidneys, leading to an eventual atrophy of the cellular elements of these organs.

A CONTRIBUTION TO THE KNOWLEDGE OF SPLENIC ANEMIA. Ralph G. Stillman, New York.

Abstract. A case is described in which splenectomy was performed seven years after recognition of splenomegaly. After operation hemorrhages ceased for six years and then recurred so that a second operation was performed. At the second

operation the liver was found to be normal and the splenic vein thrombosed and canalized. The normality of the liver argues for the theory that the disease is located primarily in the spleen. The occurrence and recurrence of hemorrhages can be explained on the basis of changing circulatory conditions.

Intestinal Obstruction and Pernicious Anemia. Report of a Case. Russell L. Haden, Kansas City, Kansas.

Abstract. True pernicious anemia is a clinical entity seemingly due to a specific toxic agent. The clinical evidence suggests that the toxin arises within the gastro-intestinal tract. A blood picture simulating pernicious anemia has been observed following gastrectomy, in malignant disease of the stomach or colon and with parasitic infestation of the intestinal tract. It is possible that in these various conditions there is present a common toxin which may be identical with that giving rise to true idiopathic pernicious anemia. The occasional occurrence of the clinical picture of pernicious anemia with chronic intestinal obstruction is further evidence suggestive of the gastro-intestinal origin of a hemolytic toxin. Seyderhelm noted a rapid improvement in cases of pernicious anemia after an ileostomy. Giffin and Dixon have reported similar findings. Seyderhelm has produced a megalocytic anemia in the dog by stenosing the ileum. I have recently observed a patient who presented some points of interest. A woman, 65 years old, with negative past history showed a typical blood picture of pernicious anemia. No free hydrochloric acid was present in the gastric contents. Under treatment there was marked improvement. She then developed signs of a progressive intestinal obstruction and died. At autopsy there was a marked obstruction of the duodenum and ascending colon due to a malignant growth of the gallbladder. The anemia may have been a coincident condition. It seems most probable, however, that the anemia which was clinically indistinguishable from idiopathic pernicious anemia was due to a hemolytic toxin arising in the intestinal tract. With this possibility the case is of interest in connection with the gastro-intestinal origin of the toxin of pernicious anemia.

Studies of the Blood in an Epidemic of Smallpox. Kano Ikeda (by invitation), Minneapolis.

Abstract. The mild pustular type shows a definite leucopenia with the initial rash, and the maximum leucocytosis during the pustular stage. Relative neutropenia is constant.

The severe pustular type shows a progressive leucocytosis with a relative neutrophilia during the initial stage and the period of desiccation. A terminal lymphocytosis occurs in fatal cases. In both pustular types the platelets are low at first but increase rapidly after the vesicular stage.

In the purpuric type there is a progressive decrease in platelets and neutrophiles with a persistent hyperleucocytosis. Normoblasts and disintegrated leucocytes are found with normal hemoglobin and red cell count.

Rheumatic Endocarditis as Related to Subacute Bacterial Endocarditis. B. J. Clawson, Minneapolis.

Abstract. Eighty cases of subacute bacterial endocarditis are compared with thirty-five cases of rheumatic endocarditis in respect to (1) blood picture, (2) anatomic characteristics and (3) the bacteriology. A close pathological and etiological relationship is evident. The two types clinically are distinct because of anatomical variations. These differences seem to depend upon the character

of the inflammation (whether or not primarily proliferative), the degree of valvular involvement, and the severity and duration of the process. Rheumatic endocarditis is characterized by a proliferative type of inflammation while the subacute bacterial endocarditis is characterized by an infected thrombus on the infected valve. From these studies it seems possible that these two forms of endocarditis represent mild and severe degrees of the same infection.

ON THE INCIDENCE OF ACUTE AND SUBACUTE INFECTIVE INFLAMMATORY PROCESSES IN CARDIO-VASCULAR DEFECTS AND ON MALFORMED SEMILUNAR CUSPS. WITH A STATISTICAL REVIEW OF THE LITERATURE AND REPORTS OF SIX CASES, IN THREE OF WHICH WAS A FUNGATING MYCOTIC ENDARTERITIS OF THE PULMONARY TRUNK.* Maude E. Abbott, M.D., Philadelphia.

Abstract. Cardiac anomalies, of a form which permit the subjects to attain adult life, are very frequently the seat of a bacterial inflammatory process which is usually engrafted on a preëxisting sclerosis at the site of the defect, which marks the seat of strain. Such cases are especially malformed semilunar cusps, and septal defects and patent ductus in which an arterial-venous shunt occurs through the defect. In 555 defects "of clinical significance," acute endocarditis occurred in seventy-eight cases (17.6 per cent). The incidence was highest in malformed semilunar cusps, 18 times in forty-eight cases (45 per cent), — in defects at the upper part of the interventricular septum, 16 times in forty cases (40 per cent); in defect at the lower part of the interventricular septum (persistent ostium primum), 6 times in thirteen cases (38 per cent); in pulmonary stenosis (mostly with associated ventricular septal defect), 20 times in eighty-two cases (24 per cent); and in patent ductus arteriosus, 15 times in sixty-seven cases (22 per cent). Acute endocarditis did *not* occur in the thirty-one cases of defect of the upper part of the interauricular septum (patent foramen ovale), thus indicating that proximity to the valvular endocardium is a predisposing cause. Nor was it present in any of the cases of extreme congenital cyanosis, as these patients usually die in the first months or years of life before the age at which an acute endocarditis is liable to supervene. In septal defects and patent ductus the lesions are practically always, and sometimes exclusively, right-sided, and frequently involve the pulmonary artery and valves in a fungating growth of extraordinary luxuriance. The infection is usually of the subacute bacterial type, produced by the non-hemolytic streptococcus (Libman), but in the right-sided lesions of cardiovascular defects, acute infection by the streptococcus hemolyticus, pneumococcus, or gonococcus, is relatively commoner than in the left-sided lesions. The localization of the vegetations in the pulmonary artery and right ventricle in these cases supplies important morphological proof that the direction of the anomalous intracardiac or intra-arterial current flow is from left to right through the defect, contrary to the conclusions drawn by Holman from analogy with the condition in arterio-venous aneurysm of traumatic origin.

Six personal observations are reported, all in adults.

Case 1. Subacute bacterial endocarditis; healed and healing stages with recent exacerbation of aortic and mitral valves and margins of large defect at base of interventricular septum with dextroposition of the aorta. *Streptococcus viridans* septicemia, embolic lesions in kidneys and myocardium.

* Published in Annals of Clinical Medicine, September, 1925.

Case 2. Perforation at upper part of interventricular septum with fused supplementary (fourth) aortic cusp and cauliflower vegetation occluding defect and protruding into right ventricle. Pneumococcus septicemia; lobar pneumonia.

Case 3. Large congenital defect at base of interventricular septum with aorta riding over it. Fungating mycotic growth completely filling pulmonary artery to its bifurcation. *Streptococcus viridans* in blood culture.

Case 4. Saccular mycotic aneurysm of the pulmonary artery at orifice of a partly obliterated ductus. Extensive vegetative endarteritis of the pulmonary artery extending down to and completely destroying the pulmonary valves. Large patent foramen ovale and hypoplasia of aorta. History of precordial pain, "bronchitis," clubbing, purpura, etc. Duration nine months.

Case 5. Patent ductus arteriosus in acute infective pulmonary endarteritis in a girl aged 19 years, dying of pneumococcus septicemia.

Case 6. Ruptured aneurysm of the right aortic sinus of Valsalva with associated interventricular septal defect and subacute infective endocarditis of margins of defect, aortic and pulmonary valves and conus of right ventricle. History of arterio-venous communication nine years, and of acute endocarditis ten months. Streptococcus septicemia.

Spontaneous Rupture of Heart Based on Thirteen Unpublished Cases and Six Hundred and Twenty from the Literature. E. B. Krumbhaar and C. Crowell, Philadelphia.

Abstract. Study of several cases of this rather rare condition that occurred at the Philadelphia General Hospital within a short period emphasized the fact that the usual text-book accounts are based on antiquated and inaccurate descriptions. As its dramatic qualities induce to frequent case reports and its rarity prevents its being observed often by a single individual, it seemed desirable to correlate our own findings with those reported in the literature of the past fifty years. In addition to the 13 hitherto unpublished cases that we report from the Philadelphia and Pennsylvania Hospitals and the University of Pennsylvania, we have analyzed 266 single case reports in some detail and added 354 further cases from collected studies of Quain, Minet and Le Clare and Robin and Nicolle, making a total of 633 cases, though of course no one item was studied in this number. Repetitions were avoided wherever known and other group reports were not utilized where repetition seemed likely. It can be absolutely excluded, however, only in the first two groups. Circumstances forbid giving more than the briefest outline of what has been a very time-consuming undertaking.

Spontaneous rupture of the heart is chiefly an accident to the left ventricle of the aged, and in the aged is practically always due to coronary disease. It most frequently occurs in an acute infarct of the anterior surface of the left ventricle following sudden thrombosis of an artery or a branch; or less frequently the infarct may follow gradual fibrotic occlusion of the lumen.

With severe coronary sclerosis and consequent myocardial degeneration (usually with more or less cardiac aneurysm), rupture may occur in an area not obviously necrotic and supplied by a patent artery. The bursting of a cardiac aneurysm has been observed, but is rare. Evidence is presented to show that the formerly popular diagnosis of fatty degeneration is usually incorrect or open to serious question.

Other rarer causes of spontaneous rupture considered in this analysis are "ulceration," fatty infiltration, fibrosis, syphilis, abscess, brown atrophy, parasitic cyst, tuberculosis, melanotic sarcoma. Most of these are based on reports of little value on account of the antiquity or incompleteness of the data.

Evidence about the site and character of the tear is considerable and accurate, but the actual mechanism which produces rupture and the actual cause of death are not clearly understood.

All classes and occupations are liable and the exciting causes are most diverse. Premonitory symptoms are frequent but not characteristic. Terminal symptoms are usually so abrupt that little treatment can be even attempted and the antemortem diagnosis is seldom made.

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A STUDY OF RABIES, WITH REFERENCE TO A NEURAL TRANSMISSION OF THE VIRUS IN RABBITS, AND THE STRUCTURE AND SIGNIFICANCE OF NEGRI BODIES *

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PART I

It has been previously shown that when a virulent neurotropic strain of the virus of herpes simplex is inoculated in sufficient quantity into the right masseter muscle of rabbits, one can demonstrate the first resulting cerebral lesion within the corresponding fifth motor nucleus. The virus affects especially the ganglion cells without demonstrable lesions in the right fifth motor nerve throughout a wide extent of its extracerebral course.[1] For this and other reasons it is believed the herpetic virus enters the brain from a peripheral focus of infection through the axis-cylinders of nerves supplying the infected area. There is also strong anatomic and experimental evidence for believing that the virus of rabies enters the brain through the nerves which supply the area wounded by the bite of a rabid animal, though the prevailing view seems to be that the virus gains entrance to the central nervous system through perineural lymph spaces [2, 3, 4, 5].

Several points of similarity between the infections of the central nervous system caused by the herpetic and the rabic viruses suggested that one might be able in like manner to demonstrate the first lesions of rabies in the ganglion cells of the fifth motor nucleus

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on the same side, if one inoculated the virus into a masseter muscle. In this way like evidence might be gained that in rabies as in herpes the virus is conducted to the brain along the axis-cylinders of nerve cells.

Both herpes and rabies induce within cells of the central nervous system structural changes which render these infections especially adaptable to cytologic investigation. Recent studies have shown that in early herpetic lesions characteristic intranuclear bodies appear, the presence of which often renders it easy to follow the course of the infection.[6,7] In the case of rabies, the presence of typical Negri bodies in cells of the nervous system has been regarded for several years as pathognomonic of the disease. Thus we have in the one case intranuclear and in the other intracytoplasmic bodies stainable by suitable methods; and it was hoped that the latter might serve like the former as guide-posts along the infected paths. For example, if one succeeded in demonstrating Negri bodies within the ganglion cells of the right motor nucleus following an inoculation of rabic virus into the right masseter muscle, and in no other cells of the nervous system and if lesions were not found along the course of the right fifth motor nerve, it might reasonably be supposed that the virus had entered these cells through axis-cylinders rather than through perineural spaces. Because the motor nuclei of the fifth cranial nerves lie deep in the pons, it can hardly be imagined that virus would affect these cells first if it were borne only by a perineural fluid.

Experiments were accordingly undertaken with this object in view. Rabbits were chosen as the experimental animal because they are very susceptible to infection with rabies; they are easy to handle; and from previous experience it was known to be feasible with their small brains to make a study of the entire fifth motor nuclei in serial sections. These considerations overbalanced the possible disadvantage that, as is well known, Negri bodies are relatively small in rabbits infected with rabies.

It was necessary to use street virus for the study in view of the established fact that in infections with fixed virus the Negri bodies are always very small and in a high percentage of cases have not been demonstrated at all. In the beginning it was a question whether one would be able regularly to infect rabbits with rabies by an injection into a single masseter muscle, as this site for intramuscular

inoculation has not apparently been tried before. The situation of choice has been into the muscles of the back on both sides of the vertebral spines, large quantities of infectious material being injected to insure a successful result. The high percentage of successful inoculations reported following the latter procedure, however, encouraged the hope that a single injection of a considerable quantity of material into the masseter muscle on one side, especially as it is relatively near the brain, would be sufficient to infect the animal. This proved to be true.

A most important consideration was to find a suitable method for staining the Negri bodies; one that could be easily applied to a number of sections at a time in as much as it would be necessary to cut the fifth nuclei serially; and one that would readily exhibit the smallest and earliest forms. The methyl blue eosin method of Mann,[8] which was employed by Negri, was tried, but it was found to be necessary to follow the original instructions of Mann and stain the sections twenty-four hours in order to demonstrate small forms. In addition to the long time necessary for staining, each section required careful manipulation. The results with tissues fixed in Zenker's fluid by injecting the fluid through the carotid arteries were disappointing in that the sections took too deep an eosin stain, requiring long decolorization in alkali alcohol. They rarely accepted sufficient blue to afford a pleasing contrast so that the bodies could be easily detected.

The eosin-methylene blue method of Lentz [9] was not so satisfactory for our purpose as was that of Mann. Besides requiring much manipulation of slides, it did not give a sharp staining of the Negri bodies with tissues fixed in Zenker's and Helly's fluids. The method is recommended especially as a means of demonstrating clearly the "Innenkörper" and since the presence of these structures has been relatively uncommon in the bodies as found in the rabbit's brain, the method was not satisfactory for the purpose in view.

The eosin-methylene blue method of Mallory [10] was employed and gave good results with Zenker-fixed tissue in staining the larger bodies, but the smaller ones either were not stained or were obscured in early stages by the deeply staining Nissl substance.

After some experimentation with various dyes, the following method was devised which has, on the whole, given much more satisfactory results than any of the others. The only deviation from

the usual procedure in preparing sections for staining was the use of a 1 per cent solution of corrosive sublimate in 95 per cent alcohol to remove the iodin after solution of the mercury precipitate in tissues fixed in Zenker's and Helly's fluids. This technic has been followed for some years and seems with the following method to heighten the selectiveness of the stain. The carbol anilin fuchsin solution has already been described by the writer.[11] The method in full is as follows:

1. Fix fresh tissues in Zenker's fluid for 24 hours. The method is not applicable to tissues fixed in Helly's fluid.
2. Stain sections cut from paraffin 10 minutes in the following solution:

Alcohol 20%	100.0 c.c.
Phenol (pure)	1.0 c.c.
Anilin oil	1.0 c.c.
Basic fuchsin	0.5 gm.

   The finely powdered or granular dye dissolves easily and the solution is immediately ready for use. Exposure to light and air causes a precipitation of the dye in about two months so that the solution becomes weaker and must be discarded. It is convenient to keep the required alcoholic solution of fuchsin, and to add the phenol and anilin oil when it is desired to make up the stain anew. If sections are stained in the solution for an hour or more they gradually become brown and useless. In our experience it is safe to stain the sections as long as one half hour but the best results are obtained within ten minutes.
3. Wash off the excess stain in running water, blot with filter paper and decolorize in 95 per cent alcohol until the sections become pink.
4. Wash off in water and counterstain 15 to 60 seconds with Loeffler's methylene blue.
5. Wash in water. Dehydrate and decolorize for a few seconds in absolute alcohol (until the excess blue is removed).
6. Clear in xylol; mount in balsam.

By this method the Negri bodies are stained a sharp, bright red against a pale blue background. "Innenkörper" are blue, nucleoli, fibrin and red blood corpuscles are red and Nissl substance blue. One can readily detect the smallest forms of Negri bodies. In the brain of a rabbit infected with rabies, minute forms are far in excess of the larger ones, especially in early stages. "Innenkörper" are not always stained by this method, but vacuoles are evident.

A strain of street virus was obtained from a dog's brain preserved in 50 per cent glycerol for four days, and probably had begun to autolyse before being placed in glycerol. About 1 gm. of this material was washed in salt solution, ground in a mortar and suspended in 10 c.c. of saline solution. Then 2 c.c. of the suspension

were injected into the right masseter muscle of each of two normal adult rabbits. Both of these rabbits developed rabies and from the brain of one, the strain of virus used in subsequent experiments was derived. The clinical history of the disease in this animal is recorded as follows:

R 1. Normal adult rabbit.

Nov. 18, 1924. Received 2 c.c. of saline suspension of dog's brain infected with rabies (street virus). The material was injected through a large needle into the right masseter muscle. When the needle was well into the muscle the injection was made a little at a time into several different places and the muscular tissue repeatedly punctured by moving the needle point about.

Nov. 20. Rectal temperature 41 C. Right cheek not swollen. Slight lacrimation of the right eye.

Nov. 22. Temperature 41.2 C. Purulent conjunctivitis of both eyes. Cornea clear. Cheek not swollen.

Nov. 24. Temperature 41.2 C. Conjunctivitis, both eyes.

Nov. 26. Temperature 40.0 C. Conjunctivitis, both eyes.

Nov. 28. Temperature 39.5 C. Conjunctivitis improved.

Nov. 30. Temperature 39.2 C. Conjunctivitis improved.

Dec. 4. Temperature 39.2 C. Emaciated, salivated, weak.

Dec. 6. Temperature 39.8 C. Very thin and weak.

It hops with great difficulty, humping its back in a peculiar manner and falling over in the attempt. On falling, the hind legs seem especially weak, remaining extended, and the animal is unable to get up. No evidence of paralysis. No infection in the nose; eyes healed; no diarrhea. Killed with chloroform. No gross pathologic changes were found in the organs at necropsy. The brain was removed aseptically and about 1 gm. from the region of the right Ammon's horn was ground in a mortar, suspended in 6 c.c. of saline solution and injected into the right masseter muscle of each of two adult rabbits, R 2 and R 3. The medulla, pons and a portion of the Ammon's horn were fixed in Helly's fluid. The remainder of the brain was preserved in 50 per cent glycerol.

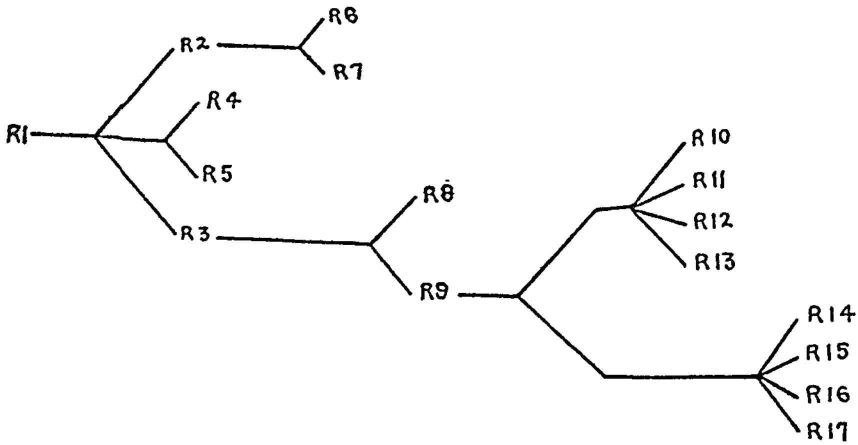
Microscopic sections from Ammon's horn and the pons show numerous Negri bodies of various sizes, often fairly large, in ganglion cells and a perivascular infiltration especially prominent in Ammon's horn.

In this animal there was evidently a primary bacterial infection associated with purulent conjunctivitis which caused the early elevation of temperature. It is interesting that no fever accompanied the symptoms of rabies. This was also true of the second rabbit receiving dog's brain. In the two animals (R 2 and R 3) inoculated with fresh brain of R 1 there was a slight elevation of temperature following the inoculation, which soon became normal and was fol-

lowed by a second rise with the onset of symptoms of rabies. In rabbits inoculated from these, there was no initial elevation of temperature but fever always accompanied the onset of symptoms usually appearing before other symptoms were apparent.

In the accompanying chart the animals used in these experiments are numbered and the source of the virus in each instance is recorded. All the animals were inoculated with a suspension of brain injected into the right masseter muscle.

All of these rabbits except one (R 7) developed rabies. This rabbit inoculated with glycerinated brain from R 2 showed no



symptoms for twenty-three days and was killed without an examination of the brain. R 6 inoculated at the same time died of rabies on the twenty-second day.

Only nine of the remaining sixteen animals were permitted to live long enough for the development of obvious symptoms other than an elevation of temperature. Of these, five showed paralysis of the front legs affecting the right leg three times, the left leg once and both legs once. There were no evidences of paralysis in other muscles. Of the remaining four animals, two were inoculated with dog's brain and ran a slow course without the development of any paralytic symptoms; one was inoculated with glycerinated brain from R 2 and ran a slow course without the development of paralysis; and one (R 9) showed a complete loss of muscular control without evidences of localized paralysis.

Thus in this small group of experiments the paralytic symptoms indicate definitely a more pronounced effect of the virus in the cervical portion of the spinal cord following an inoculation into the

right masseter muscle. This is particularly true on the side in which the injection is made but the result is not constant. In no instance, however, has a localized paralysis been observed in the hind legs.

Microscopic sections present a less definite finding and the lesions are difficult to interpret. It is clear that the injury is most marked in the pons, medulla and cervical cord, but the relative injury on the two sides seems in most instances about the same with possibly a greater severity on the right side.

Several rabbits were killed at various stages of the disease in order to study the first lesions, especially as they were manifested in the fifth motor nuclei. It was found that material from the third rabbit passage caused no preliminary elevation of temperature but fever was the first evidence of infection. Two series of animals were therefore studied, R 10 to R 17, after inoculation with the brain of R 9, to determine the earliest lesions. R 13 was killed on the fifth day after injection and before there was an elevation of temperature. R 12 was killed on the seventh day within twenty-four hours after the first elevation of temperature; R 17 on the twelfth day within twenty-four hours and R 11 on the eleventh day forty-eight hours after the first elevation of body temperature. R 12 and R 17 showed no other evidence of disease than fever. R 11 showed beginning paralysis in the right front leg. The protocol of R 9 given in Part II will illustrate the latest stages.

In addition to these animals, the protocols of which will be used to illustrate the others, serial sections were cut and the fifth motor nuclei studied in R 3, 4, 5, 8 and 9. The results conform to those described in the following protocols, although in R 3, 5 and 9 late stages were present.

R 13. Normal adult rabbit.

Jan. 5, 1925. Received 2 c.c. of a suspension of fresh brain from R 9 into the right masseter muscle.

Jan. 8. Temperature 39.0 C.

Jan. 9. Temperature 39.3 C.

Jan. 10. Temperature 39.3 C. Condition good, appears normal. Killed.

Brain injected through the carotid arteries with Zenker's solution. Serial sections through the fifth motor nuclei were stained by the eosin-methylene blue method of Mallory and the carbol anilin fuchsin method. The pons appears normal. The Nissl substance in the ganglion cells of the fifth motor nuclei is of normal appearance. No Negri bodies or other evidences of injury or infection are found.

R 12. Normal adult rabbit.

Jan. 5, 1925. Received 2 c.c. of a suspension of fresh brain from R 9 into the right masseter muscle.

Jan. 8. Temperature 39.2 C.

Jan. 9. Temperature 39.3 C.

Jan. 10. Temperature 39.3 C. Condition good.

Jan. 12. Temperature 40.4 C. No lameness, paralysis or other evidence of disease. Killed.

Brain injected with Zenker's fluid. Serial sections through the fifth motor nuclei were stained with eosin-methylene blue (Mallory). The cells appear in every way normal. No Negri bodies found.

R 17. Normal adult rabbit.

Jan. 27, 1925. Received into the right masseter muscle 2 c.c. of brain suspension from R 9 (twenty-two days in glycerol).

Jan. 30. Temperature 39.5 C.

Feb. 3. Temperature 39.3 C.

Feb. 4. Temperature 39.6 C.

Feb. 5. Temperature 39.5 C.

Feb. 6. Temperature 39.4 C.

Feb. 7. Temperature 39.4 C.

Feb. 8. Temperature 39.2 C. Appears normal.

Feb. 9. Temperature 40.3 C. Appears normal. Killed.

Brain injected with Zenker's fluid. Microscopic sections were made through the frontal lobes, midcerebrum including Ammon's horns, the brain stem, cerebellum, medulla and cervical cord. In addition, serial sections were made through the fifth motor nuclei in the pons. Sections were stained by the carbol anilin fuchsin method.

Very minute Negri bodies, or "lyssa bodies" according to the distinction made in the second part of this paper, are found sparsely distributed in ganglion cells of each part of the brain and cord, excepting the cerebellum. They are not demonstrable in the cells of Ammon's horns. Nissl substance is well stained generally.

The "lyssa bodies" are most numerous in many ganglion cells of the fifth motor nuclei. They are very small, homogeneous spheres and appear to involve more cells on the right side than on the left, but the difference is only relative.

Although the bodies are more numerous and in more cells in the fifth nuclei, there is already an early perivascular infiltration in the neighborhood of the tenth nuclei in the medulla and about ventral blood vessels in the cervical cord. The inflammatory exudate is not found elsewhere.

This case demonstrates the rapid spread of the virus through the central nervous system once it has entered, and convinces one of the futility of using the Negri bodies with this strain of virus as an in-

dex of the earliest site of infection in the brain and cord. It seems evident that by the time the bodies become demonstrable, the virus has already spread perhaps throughout the nervous system.

R 11. Normal adult rabbit.

Jan. 5, 1925. Received into the right masseter muscle 2 c.c. of an emulsion of fresh brain from R 9.
Jan. 8. Temperature 39.3 C.
Jan. 9. Temperature 38.9 C.
Jan. 10. Temperature 39.5 C.
Jan. 12. Temperature 39.6 C.
Jan. 13. Temperature 39.2 C.
Jan. 14. Temperature 39.2 C.
Jan. 15. Temperature 40.1 C. Appears normal.
Jan. 16. Temperature 40.2 C. Eats. Right ear hangs down. Head inclines slightly to the right. Easily excitable. Cannot support itself on the right foot which is definitely lame. Killed.

Brain injected with Zenker's solution. Serial sections through the fifth motor nuclei were stained by the carbol anilin fuchsin method.

There is a severe inflammatory reaction characterized by perivascular and tissue infiltration throughout this series of sections involving to about the same degree on each side of the pons, only the motor and accessory motor nuclei. Both sensory roots of the fifth cranial nerves are also inflamed to the point where Schwann's sheaths appear, more conspicuously on the right side where perivascular and focal cellular infiltrations are noted especially prominent just central to the sheaths of Schwann.

Negri bodies are very numerous in the ganglion cells of both motor and accessory motor nuclei, but are not found elsewhere in these sections. Most of them are quite small, appearing as spheres or ovals and often containing vacuoles. No Negri bodies are found in the cerebellum just above the pons.

Discussion

An examination of the above protocols shows that it has not been possible to prove by the methods used that Negri bodies appear first in the ganglion cells of the right fifth motor nucleus following an inoculation of the virus of rabies into the masseter muscle on the same side. It is evident, however, that an inoculation of fairly large quantities of the virus into a masseter muscle causes an infection of the brain in a high proportion of cases. With the strain of virus used, infection took place within a relatively short time, shorter

with fresh rabbit's brain than with dog's brain or glycerinated rabbit's brain.

From an anatomic standpoint there is no conclusive evidence that the virus enters the brain through nerves. The clinical course of the disease on the whole, however, may be best explained on the theory of a neural transmission of the virus from the site of inoculation, an idea founded upon much careful anatomic and experimental work. In five cases out of nine permitted to live long enough for paralytic symptoms to appear, paralysis in the fore legs was evident in three on the right side, in one on the left and in one on both sides. No local paralytic involvement of the hind legs was observed in any of these experiments. In one animal of the nine, no localized paralysis but a general loss of muscular coördination was observed. In three there was late general muscular weakness but no complete loss of control. One of the nine rabbits showed no evidence of rabies.

The paralytic symptoms, therefore, show that following an inoculation into the right masseter muscle, the anterior extremities are first affected and more often on the right side.

Histologic evidence based upon the presence of exudate, Negri bodies and degenerative changes in ganglion cells indicates that the virus, when it once enters the central nervous system, spreads with great rapidity. So far as the pons is concerned, the lesions may be relatively more pronounced on the right side and in the motor nucleus, although both sides were always involved when demonstrable changes were found.

In R 17, killed within twenty-four hours after the first elevation of body temperature, all the "lyssa bodies" found were of the smallest form. Their distribution indicates that even in this early period of the disease the virus had spread possibly throughout the central nervous system. This animal presented no other symptom than fever. In this case, one also observed the greatest evidence of inflammation to be situated in the medulla and cervical cord, although the "lyssa bodies" were apparently more numerous in the ganglion cells of the fifth motor nuclei. It has been usual in these experiments to find the severest lesions in the pons, medulla and cervical cord. This experience is in opposition to the view that the base of the cerebrum, that is Ammon's horns, is the site of predilection for the virus of rabies as indicated by Negri bodies. A similar view was also held regarding the localization of herpes virus, but it has been

conclusively shown that if the virus of herpes simplex enters the central nervous system through one of the peripheral nerves, the lesions at the base of the brain are always secondary and represent an extension of the virus from a lesion at the entrance of the peripheral nerve involved. This appears probably to be the case also with this strain of lyssa virus. It is true that in the first rabbit passage lesions seemed most marked in Ammon's horns. In this rabbit the progress of the disease was relatively slow and the symptoms mild. The virus had not, one might suppose, become well adapted to the new host and time was given for well marked lesions to appear at this point of secondary distribution. However, when more virulent rabbit's brain was used for the injections, the lesions were always more marked in the pons, medulla and cervical cord, which is the segment presumably nearest the point of entrance of the virus.

It is to be noted that in every case in which sections were made through the fifth motor nuclei, "lyssa bodies" were present in the ganglion cells, and if the virus entered the brain only through the right fifth motor nerve its course toward the spinal cord was much more injurious and destructive than its progress toward the cerebrum. The relatively more intense inflammation of the medulla and cervical cord suggests that the cells of these parts are more susceptible to injury by the virus, or that the virus may enter this region through sympathetic nerves. Unfortunately the cervical ganglia in these cases were not studied, but the sympathetic innervation of the muscle inoculated affords a possible portal of entry into the cervical cord if the virus is capable of progressing along axis-cylinders.

In favor of the passage of the virus along axis-cylinders, it seems to us, is the fact that in all except the very earliest cases there is evidence of injury in the central portion of the sensory division of the fifth cranial nerves. In earlier cases the cells are more or less extensively infiltrated and especially just internal to the plane where Schwann's sheaths appear. This lesion is always bi-lateral but more pronounced on the right side. In the latest stages disintegration of axis-cylinders is seen in this segment of the nerve. In one instance a loss of substance and a focal cellular infiltration occur. Coincidently there appears a very destructive lesion in the corresponding Gasserian ganglion proportional in extent to the in-

volvement of the sensory root. This lesion was studied in five cases including early and late stages. In all stages there is necrosis of ganglion cells and in the latest stage almost complete destruction of these cells, although in the early cases no evidence of inflammation along the extracranial course of the nerve is found.

In the most advanced case, various degenerative changes in axis-cylinders and a perivascular infiltration are seen in the nerve in the neighborhood of the ganglion. Evidences of injury always appear first within the ganglion cells, not in the surrounding tissue. These cells may undergo complete necrosis without cellular or other exudate about them. The inflammatory reaction which later occurs is focused about individual injured cells. These ganglion cells are surrounded by a mantle of cells whose cytoplasm and fibrils enclose them and seem to protect them much in the same way that the myelin sheaths isolate the axis-cylinders. Yet it is not the surrounding cells that show the first evidences of injury. It is known that the virus is present in such ganglia. Negri bodies are demonstrable in many of the cells, as Negri himself first demonstrated after inoculating the virus into the brain. It seems much more probable that the virus proceeds from the brain along the axis-cylinders of the sensory division which offers such a broad expanse in the pons and medulla for receiving an infection than, as suggested by Koch, that in a hypothetical stage of generalized infection the virus localizes in the Gasserian ganglia as it does in the brain. The lesion in these ganglia is in our experiments much more extensive than has been observed in other cranial or spinal ganglia, indeed they are the only ganglia in which necrosis of nerve cells is found.

These experiments illustrate the fact that Negri bodies or "lyssa bodies" may occur in great numbers within ganglion cells entirely in the absence of evidences of inflammation in the form of cellular or other exudation. It seems quite evident that cellular exudation is dependent upon the degree of injury produced by the virus and is not a necessary accompaniment of its presence. The presence of Negri bodies is an earlier manifestation of the activity of the virus than cellular exudate, yet these bodies in turn appear only after the virus has spread far to other parts. Indeed Negri bodies may persist for a relatively long time within ganglion cells without causing necrosis. Thus the action of the virus upon the cells of the fifth motor nuclei is relatively slow and mild as compared with its effect

upon the nerve cells of the Gasserian ganglia. The bodies may be present in the fifth motor nuclei at the time of the first appearance of fever, yet after three or four days of fever and shortly before the animal is about to die of the disease, it is rare to find actual necrosis, though degenerative changes may be marked. In the Gasserian ganglia, however, most of the cells die without the appearance of Negri bodies. This is in striking contrast to the action of the virus of herpes simplex. This virus rapidly causes necrosis of the ganglion cells which it first attacks, so that it is possible to find, for instance, almost complete necrosis of the cells in the right motor nucleus following an inoculation of the virus into the right masseter muscle before demonstrable lesions appear in the left nucleus or elsewhere.

Thus it appears that the presence of Negri bodies is the result of the action of the virus upon the cell and is not an essential feature of the disease. One may see cells both in the brain and in the Gasserian ganglia which show all degrees of disintegration and not infrequently necrosis, yet in the absence of Negri bodies, as has been frequently observed by other writers. In fact, in the Gasserian ganglia where the virus is most destructive, these bodies are relatively scarce in comparison with portions of the brain where necrosis is rarely observed. The cells of the brain, for example the ganglion cells of the fifth motor nuclei, evidently offer a much stronger resistance to the action of the virus than those of the Gasserian ganglia and perhaps than those of the ventral horns of the cervical cord. This very resistance may be responsible for the development of Negri bodies in greater numbers. The impression has been gained that the various structural changes observed in ganglion cells including Negri bodies are the result of the action of the virus upon the cell and the reaction of the cell to the resulting injury. It is believed that neither the virus itself nor a characteristic structural change which may be regarded as a constant accompaniment of the virus has yet been demonstrated.

The material which was prepared for this study has been utilized in a cytologic investigation of the nature of the cellular changes with especial reference to the structure and significance of the Negri bodies. The results are recorded in the following paper.

PART II

Before Negri described the bodies, which now bear his name, in the cells of the nervous system of animals infected with rabies, several investigators had noted various degenerative cellular changes associated with the lesions. These were regarded as general in their nature, that is to say, non-specific for the disease. After Negri's [12] very valuable discovery many observers believed with him that one had now in an easily demonstrable form at least a phase in the developmental cycle of the true parasite of rabies, protozoan in character. Subsequent studies, however, have gradually undermined Negri's hypothesis until nowadays perhaps no one believes that these structures, whose important practical significance is everywhere recognized, are really protozoa. However, many students of rabies, perhaps most, have not disassociated the Negri bodies from what they believe to be a visible form of the real virus. That is, they consider them structures with which the virus is in some way actually united, giving to them a specific morphology. Thus Volpino,[13] Babes [14] and adherents to the clamydozoan hypothesis hold that the minute basophilic structures within the Negri bodies constitute the specific portion and represent the true virus, while the eosin-staining portion is a sort of capsule probably produced by the cell as a hyaline mantle by which the virus may be in a measure isolated. These "Innenkörper" and the vacuoles in which they lie were carefully studied by Negri and considered by him to be a part of the internal structure of his protozoön.

Another view, perhaps less strongly supported though no less clearly defined, is well expressed by d'Amato [15] and by Lentz.[16] They maintain that the Negri bodies are purely degenerative in nature, that is, they are the product of the action of the virus upon the cellular substance but bear no essential structural relation to the virus itself.

When this study was undertaken we had no preconceived view regarding the essential nature of Negri bodies other than that their presence was considered pathognomonic of the infection. After a short study, however, of the eosin-stainable structures in the ganglion cells of rabbits infected with rabies, it became a serious question what could safely be regarded as a Negri body and what not; for it was very evident that the majority of these cytoplasmic

bodies conforming in other respects to those described by Negri were quite hyaline and apparently homogeneous and presented no "Innenkörper" stainable by the methods ordinarily used for their demonstration.

Negri himself warned that if the bodies were too deeply stained with eosin by the Mann method they often appeared homogeneous, but with a little experience in differentiating the sections an inner structure could be demonstrated. Yet he seems to have had his difficulties in this respect for in his illustrations which were reproduced presumably from characteristic preparations, one sees apparently homogeneous bodies without central vacuolation along with others which exhibit the typical structure. These homogeneous forms are especially numerous in his preparations from the brain of rabbits. Lentz emphasizes the difficulty of demonstrating "Innenkörper" by the Mann method and devised an eosin-methylene blue stain to detect them with greater precision. Schiffmann [17] recognized three types of Negri bodies, the members of one group including the smaller forms which were homogeneous and contained no demonstrable inner structure. Finally d'Amato, using a most refined technic for the demonstration of "Innenkörper," has described in the dog Negri bodies which were quite uniformly hyaline. Babes recognized similar forms.

In our preparations from the rabbit's brain one may find in the same section typical bodies with vacuoles and central "Innenkörper" and others of equal size, sometimes in the same cell, which are apparently homogeneous. It was thus necessary to admit that in rabies there occur, in addition to the bodies with "Innenkörper," other forms in all other respects similar, yet not having this differentiation. But it did not seem justifiable to group these undifferentiated forms with true Negri bodies, as others have done, in view of Negri's original description and the practical as well as theoretical importance of the distinction.

One must judge from Negri's papers that he himself regarded the presence of "Innenkörper," or the vacuoles in which they lie, as essential constituents of the type which he considered the protozoön of rabies. However, he admitted, perhaps because of the filterability of the virus (Remlinger),[18] the probability of smaller forms which could not be distinguished, owing to their size, from secretion granules or products of cellular disintegration. But for the larger struc-

tures on which his studies were based the presence of "Innenkörper" or corresponding vacuoles was essential. This is further demonstrated by the fact that in studying the Gasserian ganglia of rabbits infected with rabies, he encountered the difficulty of finding, in addition to the characteristic types, bodies which stained with eosin by Mann's method but which were irregular in size and shape containing apparently no "Innenkörper." Consequently, he was of the opinion that these more or less irregular eosin-staining structures were degenerative products of the cell, perhaps from Golgi's apparatus. These forms have also been observed by us and will be referred to later.

An exact definition of Negri bodies is an extremely important practical point in as much as their presence is used routinely to diagnose rabies in suspected animals. Fortunately the practical difficulty is not so great as it might seem, because in the brain of dogs infected with rabies larger forms of the bodies with conspicuous "Innenkörper" are usually in evidence. The necessity for demonstrating in each case the vacuolated character of suspected bodies or the presence of "Innenkörper" within them, is illustrated by the descriptions by Standfuss [19] and by Lentz [16] of structures in the brain of dogs with *Staupe*, similar to Negri bodies, excepting that they are homogeneous and occur extracellularly as well as intracellularly.

Thus for practical reasons and from the descriptions given by Negri himself one is forced to conclude that an accurate definition of Negri bodies must include the presence of "Innenkörper." According to such a definition it was evident that the great majority of the eosin-staining bodies demonstrable in the nervous system of rabbits infected with our strain of virus were not Negri bodies. For present convenience they have been termed "lyssa bodies." They have all the characteristics of Negri bodies excepting a demonstrable inner structure. It is believed, as will develop later, that "lyssa bodies" are in all essential respects similar to Negri bodies except for a different morphologic configuration.

The "lyssa bodies" are to be distinguished from the eosin-staining granules described by d'Amato in ganglion cells in cases of rabies and other conditions. These amorphous granules have been observed in this series of rabbits. They are of approximately uniform size and are arranged in the cytoplasm in rather dense groups often

at the pole of a cell or near the nucleus. These granules seem to be as d'Amato suggests, an early stage in the formation of pigment. The "lyssa bodies" are also to be distinguished from *Staupe* bodies by their intracellular situation and association with Negri bodies and the virus of rabies. They vary greatly in size, but in our experience both the smallest and the largest cytoplasmic bodies found in rabbits belong to this group. The smallest are spherical, while the larger ones are often irregular in shape with smooth surfaces sometimes penetrating into the cellular substance as if a molten material had been poured into the cytoplasm. They stain in every respect identically with the eosinophilic substance of Negri bodies by the methods of Mann, Lentz and the eosin-methylene blue method of Mallory, but they have been most easily and sharply stained, as have also the Negri bodies, by the carbol anilin fuchsin method described in the first part of this paper. This method has been used especially in the study of their origin and significance.

The distinction between Negri bodies and "lyssa bodies" has been emphasized because it has been possible to demonstrate a degenerative change in certain ganglion cells affecting the neurofibrillar apparatus in the tissues at our disposal previously fixed in Zenker's solution and stained by the carbol anilin fuchsin method. This degenerative change results in the formation of bodies identical in size, shape and staining property with "lyssa bodies." It has also been possible to observe the initiation of structural transformations in the mitochondrial substance of the cells and their processes which make it seem very probable that both Negri bodies and "lyssa bodies" have a common origin in degenerative changes in the mitochondrial and neurofibrillar apparatus of the cell, and are in no essential respect representative of the actual virus.

The changes in the neurofibrillar apparatus are found in the pons especially affecting the large ganglion cells of Deiter's nuclei. The animal in which they occurred ran a stormy course and was permitted to live until almost moribund so that the most extreme lesions might be studied. The protocol is as follows:

R 9. Normal adult rabbit.
Dec. 24, 1924. Received into the right masseter muscle 2 c.c. of an emulsion of fresh brain from R 2.
Dec. 29. Temperature 38.8 C.
Dec. 30. Temperature 39.0 C.
Dec. 31. Temperature 39.7 C. Appears in every respect normal.

Jan. 2, 1925. Temperature 40.3 C. Weak and somewhat unsteady but not lame.
Jan. 3. Temperature 40.3 C. Very unsteady.
Jan. 5. Temperature 38.7 C. Extremely weak; complete loss of muscular coördination; cannot stand, falls to the right; struggles. Fresh corneal ulcers; some salivation. Killed with chloroform and exsanguinated.
Brain removed aseptically and placed immediately in Zenker's solution.

Microscopic sections, serially through the pons including the fifth and eighth nuclei, were stained by the carbol anilin fuchsin method.

There is a moderate general perivascular infiltration most marked about the large vein through the accessory fifth motor nucleus on each side. In the ganglion cells of the fifth motor nuclei are numerous Negri bodies and "lyssa bodies" usually quite small. These bodies are found in ganglion cells quite generally throughout the section and to an equal extent on each side. The Nissl substance as a rule is fairly well preserved and stains sharply. There is a very marked destruction of axis-cylinders and a cellular exudate in each sensory root of the fifth nerves. Negri bodies are numerous in the cerebellum above the pons.

The most significant changes in this case are found in the sections through Deiter's nuclei. Most of the large ganglion cells which receive fibers from the vestibular ganglia show considerable granulation and a pale staining of their Nissl substance. However, only an occasional "lyssa body" is to be found except in certain cells. The remarkable feature is that in many cells in each Deiter's nucleus there is evidence of a very acute necrosis, with what appears to be a coagulation of material in the cell body and all its processes. There appears as a result a network of fibrils staining deeply red and having the form and arrangement of neurofibrils. Various stages of this curious cellular change may be observed. In the first stage one can see deeply red-staining, often anastomosing fibrils occupying the center of a large dendritic process surrounded by a thin zone of granular basophilic material resembling the granular cytoplasm within the cell. As one follows the dendrite centrifugally the axial fibrillar cord soon resolves itself into a thin, discrete, deeply staining thread, eventually so thin, as the distance from the cell increases, that it becomes barely visible with highest magnification. Following this change into the cell one observes the group of anastomosing fibrils occupying the dendritic process spread in fan-shape into many smaller fibrils penetrating the cytoplasm. These

fibrils are largest and apparently most numerous about the periphery; in the interior about the nucleus they are very fine and may appear interrupted. The change is a uniform one affecting all dendritic processes alike. The network of fibrils within the cell seems to have contracted similarly to fibrin in a clot. The network is drawn away from the periphery of the cell leaving a distinct zone containing no fibrils and being partly empty and partly filled with basophilic granular cytoplasmic material of which strands and clumps still adhere to the original cellular margin. The Nissl substance is no longer recognizable as such, but perhaps it is represented by the basophilic finely granular, ground-substance in which the fibrils lie. The nucleus is often indistinctly outlined. Usually one can see an amorphous mass of blue-stained material (*Kernkappe?*) situated at one side and upon the nuclear membrane. The volume of the nucleus is diminished; the nucleolus is well preserved but tends to stain basophilically; and basophilic granules appear within the nucleus (Fig. 1).

In what one may regard as a somewhat more advanced stage, the fibrils in the dendritic processes are still clearly seen, but within the cell body they are broken into fragments and very small, often spherical structures (Fig. 2). A few fibrils about the periphery are more intact. It seems evident that in such a cell there was a preliminary contraction of the network, since the peripheral margin of fibrils is drawn away from the outer cellular border with granular basophilic material and clear spaces between. In some cells the earliest visible fibrils are extremely small and threadlike, in others they are much coarser as if several fine fibrils had cohered.

In the latest stages observed, the entire network of fibrillar material has become broken up throughout the whole extent of the cellular processes as well as in the cell body into round or oval, sometimes elongated, masses always with rounded contour (Fig. 3). These bodies vary greatly in size. Within the bundle of axis-cylinders entering the pons from the vestibular ganglion, which is preserved in the sections, one can observe with the greatest clearness the various stages in this interesting change as it affects the axis-cylinder (Fig. 4). Most of the axis-cylinders are entirely unstained. In those that are altered, it is first to be observed that they retain a pink coloration, and in the same axis-cylinder a succession of changes may be followed through a short segment to complete breaking up

into red-stained balls. The normally unstained portion shades into pink, then into a deep red disclosing several apparently anastomosing fibrils. The red fibrils seem to melt together forming a single deeply stained fiber, sometimes incorporating clear spaces corresponding to the meshwork of the preceding stage. There is finally complete coherence, then irregularities appear and the fiber breaks up, or rather seems pulled apart, forming spheres and oblong rounded masses. At this final stage one can discern a delicate unstained membrane which seems to be the remnant of a sheath of the axis-cylinder within which, as in a delicate tube, the balls and masses are arranged at close intervals along its course. Occasionally the axis-cylinder breaks up into innumerable minute bodies, though this is not common.

Throughout the cross-section of the pons at this level one can find numerous cellular processes of various sizes from large dendrites or axis-cylinders to delicate thin threads representing terminal processes which present the above described change. The different stages of it may be followed to complete breaking up into spheres or granules corresponding in size to the process from which they came. The granules and balls are often numerous and may appear to lie free within the gray substance extracellularly, but one can always satisfy oneself that they all arise through the destruction of a ganglionic process.

The change is most conspicuous in the bundle of axis-cylinders from the vestibular ganglion on each side. Many of these axis-cylinders are affected and stain sharply, contrasting in a striking manner with the neighboring unstained processes. Only the central terminal portions of these axis-cylinders are affected, and the change does not extend quite to the periphery. The extrapontine portion of the nerve appears superficially normal.

There are great numbers of "lyssa bodies" of various sizes in cells throughout this level of the pons. Their staining properties so far as has been observed, are identical with those of the altered fibrils and axis-cylinders as well as the bodies to which they give origin. With Mann's stain, the eosin-methylene blue method of Mallory, Giemsa's solution and with acid fuchsin-methyl green, the two substances stain precisely alike. No vacuoles or "Innenkörper" have been demonstrated in the bodies that result from the disintegration of the neurofibrillar apparatus.

With the carbol anilin fuchsin method the normal neurofibrils do not stain, but in the presence of the above mentioned degenerative change fibrils having the arrangement and general appearance of the neurofibrillar structure, as revealed by the Bielschowsky method, become intensely stained by the fuchsin. They do not appear precisely like normal neurofibrils in that in dendritic processes and sometimes about the cellular periphery they are thicker and give the impression that they are melting together and anastomosing. The nature of this change is not clear, and no description of such a transformation of the neurofibrillar material has been found in the literature. Significant and apparently related alterations of the fibrillar substance associated with rabies have, however, been described.

Bielschowsky[20] states that neurofibrils are very easily affected by a variety of injurious influences and he has classified under three headings the pathologic changes in this substance, which may occur under various degenerative and inflammatory conditions of the central nervous system. These groups are an increase in volume of the fibrils, fragmentation or granular disintegration and a hyalinization of the cellular body in which fibrillar material apparently becomes converted into a homogeneous substance. In none of these degenerative changes is there a similarity to the above described alteration associated with rabies, in which the fibrillar material of both the cell body and its processes becomes converted into structures so like the Negri bodies. Bielschowsky describes these changes as affecting principally and usually only the fibrils within the cell, and emphasizes that those of dendrites and axones resist injurious influences much longer. Nor do descriptions of degenerative changes in the primitive fibrils of axis-cylinders following section of a nerve, which were studied most carefully by Mönckeberg and Bethe,[21] offer an analogous series of events. In as much, however, as these studies were made with a different staining method it was thought best for the purpose of comparison to restudy the degeneration of axis-cylinders in rabbits in tissues fixed in Zenker's solution and stained by the carbol anilin fuchsin method. The sciatic nerves of four rabbits were accordingly cut and the peripheral and central ends were studied after two, four, six and nine days. The changes correspond to those described by Mönckeberg and Bethe and by others, that is, a granular disintegration and fragmentation, and in no way reproduce the appearances found in rabies.

Thus, so far as the literature of the subject has been reviewed, the degeneration of the neurofibrillar substance of the cell above described has not previously been noted. Its especial significance for the pathology of rabies rests in the fact that this material under the influence of rabic virus may be transformed into bodies individually identical with "lyssa bodies" and with the eosinophilic substance of Negri bodies. The cells, however, in which this change has been observed appear to be necrotic and it is a transformation which affects the entire neurofibrillar material of the cell, so that in this very essential respect the bodies which are formed differ collectively from those that lie within the cytoplasm of apparently living cells.

This complete transformation, however, is not the only change that affects the neurofibrils in the ganglion cells of rabbits with rabies. There is evidence that only a part of the fibrillar substance within the cell body may be so changed and converted into "lyssa bodies" by a similar transition. This evidence is based, it is true, on the ever-treacherous transition forms, but it is believed significant nevertheless. Bielschowsky describes a partial granular degeneration of neurofibrils affecting definitely circumscribed areas within the cytoplasm under other pathologic conditions.

Using the silver method Ramon y Cajal [22] and Babes have studied the neurofibrillar material in rabies and have described very distinct changes usually associated with the severer degrees of injury. They observed an increase in volume of fibrils corresponding to Bielschowsky's first type of pathologic alteration. This increase in volume is associated with a diminution in number of fibrils, so that it is difficult to say whether it is due to an actual enlargement of individual fibers or to a melting together of several originally separate ones (Bielschowsky). Babes found such fibrils in the anterior horn cells of rabid dogs, ten or more microns thick. He observed that they became altered in their staining property in that they failed to become impregnated with silver and were hardly recognizable as fibrils except that one could trace them into the normally staining fibrils of the cellular processes. He further noted that they sometimes became apparently completely homogeneous or dissolved in the cytoplasm. This stage probably corresponds to Bielschowsky's third type of pathologic alteration. The altered staining property of the material offers a difficulty with the silver method in following

any possible transition of this substance into "lyssa bodies" since they, as well as the eosin-stainable portion of Negri bodies, do not retain the silver. Using the carbol anilin fuchsin method on the other hand, one gets the positive reproduction of the picture, that is, the swollen neurofibrils begin to take the fuchsin stain. It is thus possible to observe transitions which suggest very strongly that many of the larger "lyssa bodies" are formed by a fusion and a breaking up into a coarse network or irregular masses of the neurofibrillar material. This type of change is to be observed particularly well in cells of the accessory fifth motor nuclei and in the Gasserian ganglia where it is usual to find large bodies rather than small ones.

There is another type of alteration in the neurofibrillar substance clearly disclosed by this method and more commonly observed in the two above-mentioned groups of ganglion cells. That is the so-called "Alzheimer Fibrillenerkrankung"[23] in which coarse fibers and sickle-shaped bodies sharply stained by fuchsin appear within the cytoplasm. This change has been observed in other conditions and is thought by Spielmeyer[24] to be caused by a fusion of fibrils. In our preparations such structures have been found both in a stained and unstained condition. It is believed that they may be preëxisting in certain cells perhaps as an abnormality in the differentiation of neurofibrils and under the influence of rabies may become stainable by the fuchsin method.

It is thus demonstrated that under certain conditions in rabies the neurofibrillar material may be converted into bodies identical with "lyssa bodies." Whether this change is a specific one for rabies, that is, can occur only in the presence of the virus, it is impossible to say with the evidence at hand. However, there are indications that it may not be specific. For example, the bodies described by Lentz in the brains of dogs dead of *Staupe*, occurring both intra-cellularly and extracellularly, bear a striking resemblance to those formed in the last stages in the disintegration of the neurofibrillar substance which we have described. The size, shape, homogeneous structure and staining property are apparently identical. It seems possible that the extracellular situation of the *Staupe* bodies may be apparent rather than real, for when axis-cylinders and dendritic processes undergo the transformation described in rabbits the bodies sometimes appear to lie free in the tissue. Without careful study one might not associate them with cellular branches far from the

mother cell. It seems possible also that a similar change in the neurofibrillar material may give rise to the bodies which occur in ganglion cells of fowls infected with *Hünnerpest*, described by Kleine [25] and by Schiffmann [26] and suggesting to them a striking resemblance to Negri bodies.

The origin of "lyssa bodies" and the eosin-staining material of Negri bodies from an alteration in the neurofibrillar material seems to us very probable. Such a transformation into hyaline masses as has been described does not explain the architecture of typical Negri bodies nor account for the presence of "Innenkörper" which, in the opinion of many, constitute the essential part of these structures. One naturally asks whether the architecture of the typical Negri body and the nature of its inner structure are sufficiently characteristic and specific to enable one to disassociate them completely from the above-described degeneration products of the neurofibrillar substance and to continue to consider them perhaps as masses of the virus of rabies; or whether, admitting that the eosin-staining material may be composed of a substance originating from a cellular constituent (Babes), to regard the "Innenkörper" as representing the virus.

In considering this phase of the subject it is necessary to mention the description by Babes and by Koch and Rissling [27] of minute bodies, distinct from the bodies of Negri, which these authors regard as the etiologic agent of rabies. Babes found these structures only in the cytoplasm of ganglion cells, while the bodies of Koch and Rissling were found most numerously outside the cells. Judging from their descriptions and illustrations, the bodies found within the cells by both observers are possibly the same. Since our preparations have not shown any extracellular structures which may be considered identical with those occurring in the cytoplasm, the description of the bodies by Babes and his interpretation of them and their relation to rabies and Negri bodies will be adhered to in considering this phase of the problem.

Babes found in the cytoplasm of ganglion cells in regions where Negri bodies were few or altogether undemonstrable, minute bodies measuring about 0.1 micron and often occurring in pairs characteristically situated within a small vacuole. These bodies were best demonstrated by counterstaining a Ramon y Cajal preparation with Giemsa's stain, by which means they were colored almost

black and sharply exhibited. By the same procedure "Innenkörper" of Negri bodies were similarly stained, but they were often larger and more irregular in form. Babes was therefore of the opinion that he had demonstrated in the form of minute bodies lying in the cytoplasm within circumscribed spaces or vacuoles, the true etiologic agent of rabies. He interpreted the formation of Negri bodies as the product of a reaction on the part of the cell by which the virus became encapsulated, and the irregularities in size and shape of the "Innenkörper" as due to disintegrative changes going on in the imprisoned virus.

In our preparations from the brains of rabbits infected with rabies, bodies which we believe are identical with those described by Babes have been clearly demonstrated not only in ganglion cells but within axis-cylinders. It has been possible to throw some light, it is believed, on their origin and their relation to the formation of typical Negri bodies.

A study of the mitochondrial elements in ganglion cells and their processes and alterations of them under the influence of rabies, has enabled us to formulate the opinion that the small bodies of Babes and the architecture and content of typical Negri bodies are the result of disintegrative changes within the cell; and that they bear no other relation to the virus than that of an expression of its injurious action upon the cellular constituents.

The mitochondria of ganglion cells have not yet been sufficiently studied in their normal relations to offer a completely satisfactory cytologic background for accurate interpretation of their behavior under pathologic conditions. Their presence in normal, adult ganglion cells has even been denied by Meves, Duesberg and Hoven.[28] On the other hand Cowdry,[29] Held [30] and Spaltz [31] have been able to demonstrate them quite clearly in the body of ganglion cells, and they state also that similar structures occur along the axis-cylinders and dendritic processes, especially numerous about the end-organs of motor nerves.

It has been possible in our preparations to demonstrate structures resembling mitochondria in various types of ganglion cells. Filaments can also be demonstrated throughout the extent of axis-cylinders, corresponding in every way with those inside the cell, excepting that they are often much longer assuming the appearance of delicate fibrils of considerable length. These fibrils are, however,

not continuous and are therefore distinguishable from neurofibrils which have not been stained by the methods used. They seem in rabies to have a greater resistance to chemical injury than mitochondria are ordinarily considered to have, in that they are not destroyed by the acetic acid in Zenker's solution. Cowdry states that the mitochondria of nerve cells are destroyed by fixation in this fluid, but this has not been our experience either with normal tissue or that from rabid animals. It is true, however, that the mitochondria are ordinarily more difficult to demonstrate especially in axis-cylinders after fixation in Zenker's fluid. In the axis-cylinders from animals infected with rabies they seem to be more easily demonstrable and perhaps larger than under normal conditions. Within ganglion cells affected by the disease their number may appear greater than normal, but in later stages of the disease or in the presence of more profound injury they are greatly diminished and show various types of degenerative tranformation.

The method employed in this study especially for their demonstration in the central nervous system consisted in anesthetizing the animal with ether or chloroform, exsanguinating by cardiac puncture and fixing the brain immediately by injecting Helly's fluid through the carotid arteries. The brain was then removed and thin cross-sections (2 mm.) through the areas desired were cut and placed in the same fluid for twenty-four hours. They were removed to fresh Zenker's fluid without acetic acid, in which they remained three days; then they were washed in running water twenty-four hours and embedded in paraffin in the usual way. Sections 2 to 4 microns thick were stained by the acid fuchsin-methyl green method, or by the following technic which often gave better results with the medullated axis-cylinders:

1. Stain sections 1 minute in the carbol anilin fuchsin solution.
2. Wash in running water 1 minute.
3. Decolorize in a 1 per cent solution of acetic acid in 40 per cent formaldehyde.
4. Wash in running water 1 minute.
5. Counterstain in a saturated aqueous solution of picric acid 1 minute.
6. Wash in running water 1 minute, and repeat the entire procedure from 1 to 5, several times (usually four) depending upon the depth of coloration desired.
7. Remove the sections from the final immersion in picric acid and dehydrate rapidly in absolute alcohol. Xylol; balsam.

This method has previously been used with success in demonstrating gram-negative bacteria in tissues after fixation in Zenker's

fluid. The mitochondria appear as dark blue rods, granules or filaments against a yellowish background. The Nissl substance is unstained; Negri bodies are unstained or are light brown; and myelin sheaths are blue.

By these methods mitochondria have been demonstrated, after fixation in Helly's fluid, in ganglion cells in the pons, medulla, cerebellum, cervical cord and Gasserian ganglia, as well as in medullated axis-cylinders. After fixation in Zenker's fluid and staining with acid fuchsin-methyl green, they were also demonstrated in cells of the Gasserian ganglia and in axis-cylinders.

Ordinarily tissues fixed in Zenker's fluid are not so good for the study of axis-cylinders which are often shrunken. In one animal (R 9), however, the mitochondrial filaments and the changes to be described in them were especially well exhibited. This will form the basis of the following description, in as much as the history of this case has been given above in full. In other rabbits whose tissues were fixed by injecting Helly's fluid through the carotid arteries, identical changes were observed.

In the serial sections through the pons, the vestibular ganglion on the right side was included so that it was possible to follow the bundle of axis-cylinders from the ganglion completely into the substance of the pons. The myelinated axis-cylinders both distal and proximal to the ganglion were very well preserved. Very thin sections (2 to 4 microns) through this region were stained by the acid fuchsin-methyl green method and the following changes were observed in the cells and their processes.

All of the axis-cylinders and their myelinated sheaths appear superficially normal. In many of the axis-cylinders the rather long, sometimes wavy but always interrupted filaments lying parallel along the longitudinal axis can be very clearly seen. They seem to present a normal appearance, although with normal tissues they have not been so clearly demonstrable after Zenker's fixation. In other axis-cylinders side by side with these one can detect irregular thickenings along the threads causing the filaments to be somewhat nodular. In still others a very striking change in the filaments has occurred in that they appear partly or completely replaced by spaces (or an unstained substance) of considerable size, many times the diameter of the fibril, and of round or oblong shape often with their longitudinal poles pointed. Sometimes these polar points lead off

into a remnant of the filament. Three or four such vacuoles may be found lying together along a longitudinal axis as if a single filament had been thus affected at three different points. The margins of these vacuoles stain red against the pink background of an otherwise apparently homogeneous axis-cylinder. On their inner surface one can often see red-stained, irregular thickenings, sometimes one alone, again two with one situated at each longitudinal pole and less frequently three or more (Fig. 5, *a* and *b*). In similar preparations counterstained with Loeffler's methylene blue instead of methyl green, these bodies are stained deep blue in contrast to the otherwise red outline of the vacuole (Fig. 5, *c*). In such a preparation 1 to 2 microns thick, one can frequently trace these structures lying within red-outlined vacuoles along an axis-cylinder into the cytoplasm of a ganglion cell. Within the body of the cell they are irregularly distributed and vary in number. There may be only four or five, or as many as twenty, sometimes more. Similar structures are found in ganglion cells and axis-cylinders within the pons.

Some of the smaller vacuoles in the axis-cylinders have a relatively thick red-staining margin, and one may find here and there also in the same axis-cylinder an occasional independent mass or granule which corresponds to the thickenings observed in the more normal filaments. Stained by Mann's method the vacuoles are quite distinctly outlined by the blue stained material of the axis-cylinder. The margins may take a faint eosin coloration, but the granular bodies within are never distinct. In the nerve cells of the ganglion in which these structures appear, there are also many "lyssa bodies" of various sizes but all small. For the most part, these bodies closely approximate in size the small masses found in the vacuoles, the latter being somewhat smaller and more numerous. In the acid fuchsin-methyl green or methylene blue preparations, the two forms cannot be easily distinguished from each other unless the small bodies are seen in a distinct vacuole. The "lyssa bodies" appear as homogeneous, spherical, minute, red-staining structures irregularly arranged within the bluish cytoplasm. With the Mann or the carbol anilin fuchsin methods the small bodies of the type occurring in the axis-cylinder do not stain, while the "lyssa bodies" are demonstrable.

Identical changes in axis-cylinders especially of the fifth cranial nerves have been demonstrated in the brain and extradurally in

rabbits infected with rabies, after injecting the brain with Helly's fluid. It is often necessary to remove the chrome salts with potassium permanganate and oxalic acid before applying the acid fuchsin-methyl green or methylene blue methods, as a precipitate is otherwise apt to occur in the sections.

Such changes have not been found in the mitochondria of nerves and cells of four normal animals, in two of which the brains were fixed in Zenker's and the others in Helly's fluid. There is no indication that the changes are artifacts or the result of postmortem influences. In every instance in this study, only perfectly fresh material was used, all the animals having been killed and the tissues fixed immediately. Sections of the sciatic nerve proximal and distal to the cut ends and fixed in Zenker's fluid were studied by the same methods two and four days after section of the nerve. No similar degenerative change is observed. The axis-cylinders in these experiments underwent rather a granular disintegration, and fine fuchsinophile granules, which are interpreted as representing the remains of mitochondrial material, appear in the fragments.

One may safely conclude, it is believed, that the swelling and degeneration of the mitochondrial filaments in the axis-cylinder and presumably of similar filaments in the body of the cell are the result of the action of the virus of rabies on the living cell and its processes. It is also believed that this degeneration occurs with the formation of droplets of fluid indicated by the presence of the vacuoles, and of the small bodies within. None of the ganglion cells in which these bodies appear shows evidences of necrosis. The Nissl substance while pale is distinctly stainable and the nuclei are intact.

Mitochondria, it is true, are very susceptible to influences of an injurious nature. One finds frequently under various conditions ring-forms possibly resulting from an absorption of fluid in the process of disintegration, but never before have we observed or seen described the occurrence within mitochondrial rings of stainable material analogous to the bodies here described. Thus far the change appears to be specific. Whether these inner bodies represent transformation products of the filaments themselves or are the result of changes in the surrounding material is not clear, but the former explanation seems the more probable. They are regarded as degenerative in origin rather than as the virus of rabies. Babes thinks the virus is represented by the bodies described by him, with which, it is

believed, these inner structures are identical. We do believe with Babes, however, that they play a part in the formation of typical Negri bodies and minute "lyssa bodies." The occurrence within the same cell of minute forms of "lyssa bodies" comparable in size, number and distribution to the inner bodies as found in the vacuoles, suggests that the inner bodies may be transformed into "lyssa bodies." This takes place perhaps by a change in the surrounding neurofibrillar material so that it adheres to them and imparts an eosinophilic property. Also the individual vacuolated forms of Negri bodies are comparable to the small vacuolated structures which result from mitochondrial disintegration and which might, by a similar change in the neurofibrillar material, be transformed into vacuoles with an eosin-staining wall. For example, if degenerating neurofibrillar substance became swollen, fused and eosin-stainable, as it has been shown to be capable of becoming, and cohered incorporating the vacuoles with their inner bodies, the picture of the typical Negri body would result. Similarly, the "lyssa body" is an expression of the same process but without the presence or demonstrability of the inner bodies. According to this hypothesis the larger vacuolated Negri bodies might be formed by a coalescence of a few or many of the smaller vacuolated spheres, and in like manner some of the larger "lyssa bodies" might result from a confluence of the smaller ones. Under these circumstances one might expect to find Negri bodies composed of a mixture of the two forms. As a matter of fact one not infrequently observes in the rabbit rather large, sometimes lobulated Negri bodies, one lobe of which may be completely homogeneous while the other is vacuolated.

We agree with Babes that the "Innenkörper" of Negri bodies are probably the incorporated inner structures of the free vacuoles, for the latter correspond to them in size and show a distinct tendency to stain basophilically.

Discussion

In this series of rabbits infected with a strain of street virus of rabies by injection into the right masseter muscle, one finds necrotic ganglion cells both in the pons and the Gasserian ganglia in which no Negri bodies or similar structures are demonstrable. In the same preparations one may find other ganglion cells and axis-

cylinders which are apparently necrotic and in which the entire neurofibrillar material of the cell and its processes becomes transformed into a substance identical in staining property, so far as tested, with the eosin-stainable material of Negri bodies.

In a complete series of preparations, it has been shown that these altered neurofibrils are further transformed by fragmentation and cohesion into bodies within the cell and its processes. These bodies in gross structure and staining property are identical with what has been termed for convenience the "lyssa bodies," that is to say, bodies in every way similar to Negri bodies excepting the absence of demonstrable vacuolation and "Innenkörper." In other ganglion cells "lyssa bodies" and Negri bodies may occur side by side within the cytoplasm, but in our experience the former have greatly exceeded the latter in number. It is believed they are in reality just as specific for rabies, though for practical reasons they should not be so regarded.

In the same preparations degenerative changes in mitochondrial filaments of axis-cylinders and of the cell body are found which form structures very strongly suggesting early stages in the formation of both the vacuolated Negri bodies and "lyssa bodies." Changes are also observed in the neurofibrillar material of the cell body which seem to lead to the formation of the larger, more or less irregular, non-vacuolated "lyssa bodies," by a process of swelling, melting together and final fusion and coherence, associated with an altered staining property. In other words, all the structural changes within the nerve cells and their processes associated with rabies appear to have a degenerative rather than a specific origin, in the sense of representing the virus itself. The typical Negri bodies seem to be specific in so far as their intracellular situation and morphologic configuration are characteristic.

It is believed that the virus of rabies has not been revealed by our present methods. It is probable that it often overwhelms a cell causing it to die quickly without the formation of any characteristic cytoplasmic structure. Again it may kill the cell more gradually so that the entire neurofibrillar material undergoes a change by which it becomes readily and intensely stainable by the carbol anilin fuchsin and other methods used for the demonstration of Negri bodies. This change eventually results in a coalescence of neurofibrils and a breaking up of their substance into bodies resembling

Negri bodies. It is believed that this alteration is preceded by a disintegration of mitochondria.

In a less severe grade of injury or within cells of a higher degree of resistance, the degeneration may be only partial, manifesting itself more gradually and affecting only certain portions of the living cell. It causes in a sense a necrobiosis with the production of structures which physically may be identical with those just described but which because of a more gradual formation are more characteristic in their architecture. These forms often constitute the true Negri bodies, and they originate in a degenerative change in mitochondria which gives rise to vacuoles with bodies within them, about which through a partial disintegration of neurofibrillar material a capsule is formed.

The structure in the cell and its processes which seems to be the most sensitive to the injurious effect of the virus is the mitochondrial substance. Characteristic degenerative changes have been observed in these filaments in axis-cylinders as well as in the bodies of cells which otherwise appear relatively little injured.

The presence of this degenerative change in axis-cylinders at a considerable distance from the brain and from the mother cell is of especial interest in view of a possible axis-cylinder transmission of the virus of rabies. The occurrence of degenerative changes in the mitochondria of axis-cylinders may be interpreted in two ways.

It may be the result of an injury to the mother cell whose consequently disordered metabolism is reflected outward along its processes. The mitochondria, being especially sensitive to influences of an injurious nature, may disintegrate perhaps throughout the entire extent of the axis-cylinder. This particular type of degeneration, however, so far as we know, has not been described as occurring under the influence of toxic or other disturbances of nerve cells. If this is the true interpretation, it gives one little insight into the nature or activity of the virus, for the injury to the cell may be toxic in nature and not due directly to the presence of the virus within it.

On the other hand one may conceive of the virus itself passing along the axis-cylinder causing as it goes a disintegration of mitochondrial material. When the mitochondria are thus affected, they swell and are apparently transformed partially or completely into a fluid medium. This medium is perhaps favorable to the growth of the virus which may thereby be enabled to advance rapidly from

one level to another as the continuous formation of this favorable medium proceeds toward or away from the mother cell.

The facts at hand warrant the acceptance of the latter hypothesis, for the relatively slight evidences of injury within the ganglion cells from which these axis-cylinders proceed does not seem to be against it. Such a theory is perhaps the best explanation at hand for the fact that in the central half of the axis-cylinder processes from the vestibular ganglion in R 9, many of the axis-cylinders show the change in staining and final transformation into the "lyssa bodies" above described. Nearer to the ganglion they appear normal or show only a disintegration of mitochondrial filaments, and at the same time the mother cells are relatively slightly affected. Thus through the action of the virus the peripheral portion of an axis-cylinder may be completely destroyed, indicating that the effect of the virus may progress from the periphery inward toward the cell rather than the reverse. Under injurious influences of a general nature it has been shown (Bielschowsky) that the neurofibrils of axis-cylinders are more resistant than those within the body of the cell.

Conclusions

1. It has been possible to infect rabbits with rabies fairly regularly by inoculating them with street virus into the right masseter muscle.

2. The majority of animals thus inoculated and permitted to live long enough to develop muscular symptoms showed evidences of paralysis first in the fore legs and more often on the side of the inoculation.

3. It is believed that the virus enters the central nervous system through nerves supplying the inoculated muscle.

4. A method of staining to demonstrate Negri bodies is described.

5. A distinction is drawn between Negri bodies and "lyssa bodies," based on structural differences. "Lyssa bodies" were much more numerous in these animals than typical Negri bodies.

6. Following an inoculation of the virus into the right masseter muscle, "lyssa bodies" or Negri bodies were always demonstrable in the right motor nucleus of the fifth cranial nerve, but also in the left nucleus and widely distributed in the central nervous system. The most severe lesions, however, were always found in the pons, medulla and cervical cord, and apparently more extensive on the

right side. Ammon's horns were usually only slightly affected. Lesions in the sensory division of the fifth cranial nerve and in the cells of the corresponding Gasserian ganglion suggest an axis-cylinder transmission of the virus.

7. A degenerative change in the neurofibrillar substance of ganglion cells and their processes is described which leads to the formation of bodies identical with "lyssa bodies" and with the eosin-stainable substance of Negri bodies.

8. It is believed that "lyssa bodies "are formed directly from such an alteration of neurofibrillar material in a portion of the living cell, with or without the inclusion of inner structures.

9. Degenerative changes are described in the mitochondrial material of axis-cylinders and nerve cells leading to the formation of structures which closely resemble the "Innenkörper" of Negri bodies.

10. It is believed that the typical Negri bodies are formed within the living cell by a focal degeneration of neurofibrillar material which melts together and coagulates or coheres about one or more of the structures which result from mitochondrial degeneration. The inclusion of these bodies gives the characteristic architecture to Negri bodies which alone serves to distinguish them from "lyssa bodies."

11. The type of degeneration of mitochondrial substance in axis-cylinders and nerve cells is, so far as we are aware, specific for rabies, in that small bodies are formed which stain deeply with Loeffler's methylene blue after acid fuchsin.

12. It is believed that neither the virus of rabies nor a constant structural change associated with its presence has yet been demonstrated microscopically.

13. All the structural changes observed appear to be degenerative in origin.

14. The hypothesis is advanced that the degenerative changes observed in axis-cylinders are the result of the passage of the virus along these processes, and indicate its manner of extension from one focus to another. The material resulting from the degeneration of mitochondria may serve as a favorable medium for the growth and spread of the virus.

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EXPLANATION OF PLATES XCII–XCIII

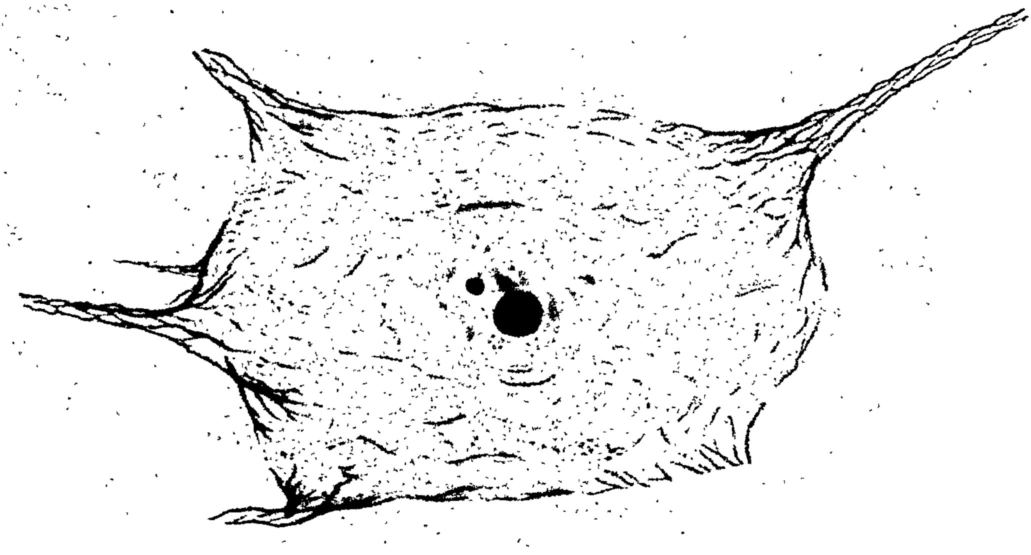
FIG. 1. Ganglion cell showing neurofibrils stained by the carbol anilin fuchsin method. The fibrillar network is evidently retracted from the original cellular margin. This is the first stage in the alteration of neurofibrils leading to the formation of structures resembling Negri bodies.

FIG. 2. Ganglion cell showing the second stage in this process. Neurofibrils are partially fragmented within the cell body, but are still preserved in the processes. The cytoplasmic bodies are in this stage very small.

FIG. 3. Ganglion cell showing the final coherence and breaking up of the altered neurofibrillar material into bodies of various sizes identical with "lyssa bodies."

FIG. 4. An axis-cylinder showing a complete series of changes resulting in the formation of hyaline bodies. The hyaline structures lie within a delicate membrane. At the extreme left, the axis-cylinder is almost completely decolorized and appears like unaffected fibers.

FIG. 5. *a* and *b* are axis-cylinders stained by the acid fuchsin-methyl green method after fixation in Zenker's fluid. *c* is stained with acid fuchsin and Loeffler's methylene blue. In *a* one can see the mitochondrial filaments, showing areas staining more deeply. *b* shows the transformation of mitochondria into globules. One can see the irregular thickenings along the inner margins. *c* illustrates the reaction of these granules to the counterstaining with methylene blue. Such dark blue dots within vacuoles could be traced along axis-cylinders into the body of the cell.



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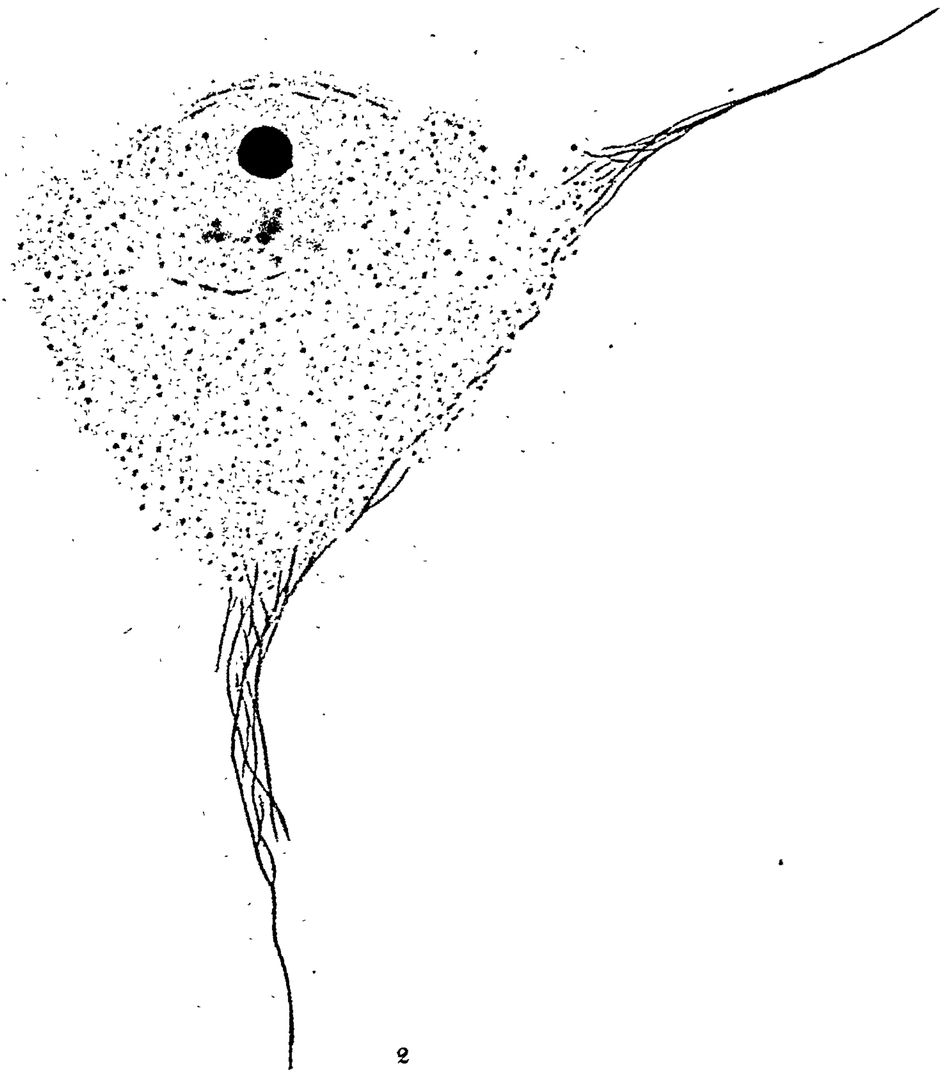
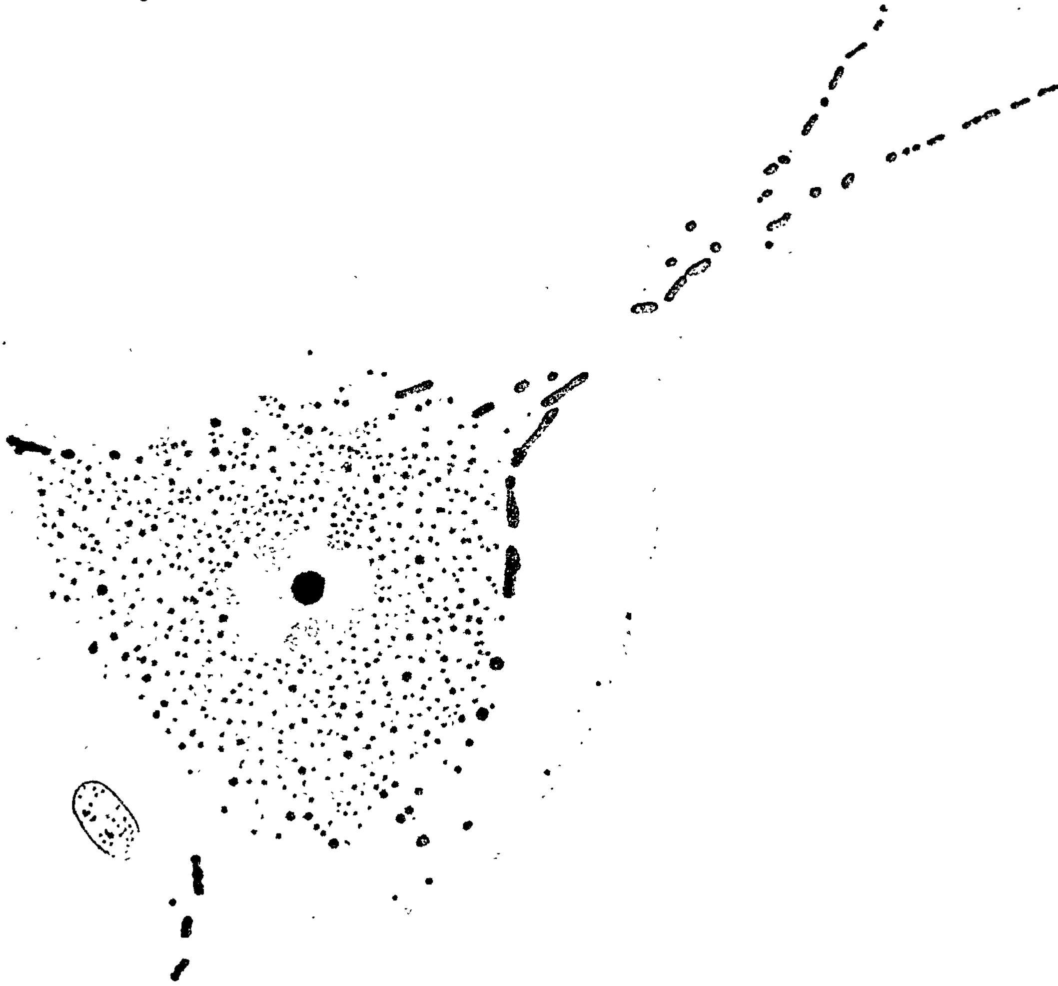


2

Goodpasture

Rabies and Negri Bodies



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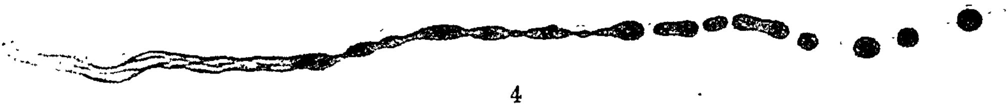

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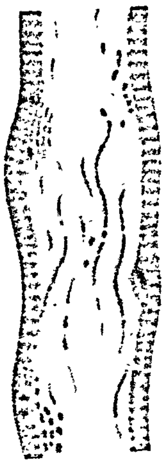

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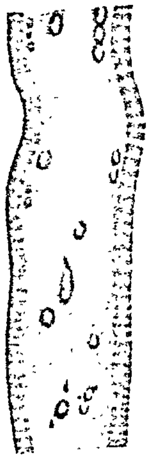

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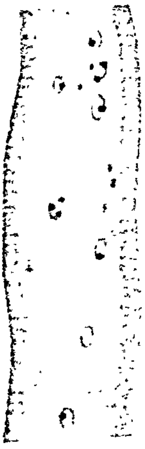

5c

Rabies and Negri Bodies

Goodpasture

DIFFUSE TUMORS OF THE MENINGES *

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One of the most remarkable examples of tumor formation is that known as diffuse sarcomatosis of the meninges. Elsewhere in the body, as implied by its very name, a tumor is made up of cells or fibers collected together to form a mass. That mass, it is true, may be flattened and widespread, as in endothelioma of the pleura or the peritoneum, but even there flat elevations or multiple nodules may be recognized.

In the leptomeninges, however, there occasionally occurs the curious condition of a neoplasm which does not give rise to a mass. A considerable number of cases of this disease have been described, but many interesting points in the pathology of the condition still remain to be cleared up.

Such terms as diffuse sarcomatosis of the meninges are somewhat misleading for they give one the conception of a pathologic entity which does not really exist. There are indeed three ways in which the condition may arise: (1) it may be secondary to some tumor outside the central nervous system; (2) it may be secondary to a tumor originating in the central nervous system; or (3) it may (possibly) exist as a primary lesion of the meninges.

Many forms of neoplasm may occasionally set up metastases in the brain, and those most prone to do so are the most likely to give rise to diffuse involvement of the meninges. The tumor may be a carcinoma or a sarcoma. Judging from the recorded cases the latter is much the more common, although it is more than probable that very many of the cases of sarcoma have no real claim to be included in this class of neoplasm. Carcinoma of the lung is perhaps the form of cancer most frequently associated with cerebral metastases. In 105 cases of carcinoma of the lung Dosquet [1] found metastases in the central nervous system in 31.4 per cent. Morse [2] has recently described an interesting case of diffuse involvement of the meninges

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in which the primary tumor was an unsuspected carcinoma of the lung only discovered at necropsy. In this and in the other instances where diffuse meningeal involvement was observed, there was a definite tumor of the brain which, as will presently be seen, probably constituted the focus from which the meninges were involved. Although the relative incidence of cerebral metastases is not so high in cancer of the stomach and of the breast, yet these diseases on account of their great frequency are those most likely to be responsible for secondary growths in the brain.

Before discussing the various forms of sarcoma which may be found in a diffuse form in the cerebral meninges and the paths by which the tumor cells gain access to the subarachnoid space, it will be convenient to present the details of a case which recently came under my observation.

Clinical History

The patient was a Galician, 41 years of age, who commenced to suffer from headaches about the middle of December, 1923. The pain came on every few hours and lasted for from ten to twenty minutes. As the headaches increased in frequency and in severity he came to the hospital on December 19th. The pain at the beginning of an attack was first felt in the forehead, but presently it passed over the top of the head and down the back of the neck. Soon the pain became constant, but still much worse in spells during which he groaned and held his head between his hands. At no time did he complain of any dizziness or tinnitus.

On admission he appeared to be in a lethargic condition, manifesting a listlessness which increased in intensity so that he lay quietly as if asleep most of the time. He could be easily aroused, however, and then answered questions quite intelligently. At this time a provisional diagnosis of encephalitis lethargica was made. The cranial nerves appeared to be normal. The eyes reacted to light and accommodation. There was no optic neuritis. The deep reflexes were unimpaired. The plantar reflex was flexor in type. The abdominal and cremasteric reflexes were present. Owing to the mental condition it was not possible to make a satisfactory examination of the sensory functions.

On the back and the anterior abdominal wall there were several small, firm, painless, non-pigmented nodules. In the right lumbar region there was a pedunculated fungating mass about 2 cm. in diameter, which had apparently been present for a number of months and which had increased considerably in size during the last few weeks.

Soon after admission to the hospital he developed stiffness of the neck and a double Kernig's sign. A lumbar puncture was done in order to exclude the possibility of syphilitic meningitis. The spinal fluid, which was not under increased pressure, was clear, showed a marked increase in globulin and contained 280 cells per c.mm. The Wassermann test was negative both in the blood and in the spinal fluid. The cells in the spinal fluid were peculiar and quite unlike any

which I had seen hitherto. They were large and pale, with a large oval nucleus and abundant cytoplasm. The sugar in the fluid was 0.05 gm. per 100 c.c.

A portion of the fungating tumor of the skin was removed and was found to be a pigmented melanoma showing numerous mitotic figures and evidence of rapid growth. A diagnosis was then made of melanoma with cerebral metastases.

The lethargy increased and the headache became worse. On January 11th the patient developed a right-sided facial paralysis. He now lay in a semi-unconscious condition all the time, the head retracted and the knees drawn up. The plantar reflex showed an extensor response on the right side and occasionally a similar response on the left side. He commenced to vomit, gradually became completely unconscious and died on January 15th.

Necropsy Findings

The *large nodule in the skin* was removed and examined with greater thoroughness than had before been possible. Part of the nodule was deeply pigmented, even to the naked eye, but other parts were completely free from pigment. The tumor cells were large and spherical with very pale cytoplasm and an oval vesicular nucleus, were arranged in indefinite alveoli and could be seen to arise in the most unmistakable manner from the deepest layer of the epidermis. The other skin nodules were of similar structure, but they presented one important difference, namely, they were situated quite deep and separated from the epidermis by a considerable interval. It appears, therefore, that they were metastatic in origin, a point of considerable importance which will be referred to in the subsequent discussion.

The *thoracic and abdominal organs* were normal with the exception of the *left kidney* which presented a small circumscribed nodule in the cortex about 1 cm. in diameter. This nodule was composed of large spherical cells most of which were non-pigmented, but some of which were loaded with fine yellow granules. In the immediate neighborhood of the nodule the outline of several glomeruli packed with pigmented tumor cells could be made out. It appears certain, therefore, that emboli of tumor cells were being given off from the fungating nodule in the lumbar region and that some of these were arrested by the glomeruli of the kidney, while others formed secondary nodules in the deeper layers of the skin.

Brain. When the brain was removed in a somewhat poor light it seemed at first to be quite normal and certainly presented no gross tumor either to the sight or touch. Closer inspection in a good light, however, showed that there was a faint darkening of the pia-arach-

noid in certain regions and associated with the darkening there was a slight thickening. What appeared to be a thin film, yellowish brown and adherent to the underlying structures, covered the greater part of the cerebrum, the brain stem and the cerebellum. The condition was most marked over the left cerebral hemisphere involving the frontal lobe as far back as the fissure of Rolando and commencing again in the occipital region; it was difficult to determine with accuracy the exact limitation. The left frontal lobe was involved to a less degree. A distinct thickening and opacity were found in the interpeduncular space and over the brain stem and cerebellum.

The brain was opened by the usual incisions but, as these did not actually pass through the gray matter, no abnormality was revealed. When, however, the cerebrum was sectioned from before backward in a series of coronal sections, a very remarkable condition at once became apparent. The actual tip of the frontal lobe appeared normal, but on passing back for 2 or 3 cm., the sections showed the pia to be not only discolored but slightly thickened. It was firmly adherent to the cortex and could not be stripped off. A change was observed at the same time in the gray matter of the cortex, which also became distinctly discolored, the gray passing into a dirty brown in striking contrast to the pure white of the unaffected white matter. At the same time it was noticed that the cortical gray matter was slightly thicker than normal, averaging from 2 to 3 mm. in width. This thickening was seen in much the most striking manner not in the superficial cortex but in the folds of the convolutions surrounding the fissures. Here the gray matter was two or three times the normal thickness and appeared to be the seat of some kind of infiltration, an appearance which was particularly striking in the occipital lobe. Both surfaces of the cerebellum showed darkening of the pia, patchy rather than uniform in its distribution.

Further examination of the cut surface under the low power dissecting microscope showed that the pigmentation did not really penetrate into the full depth of the gray matter but only for about one third the distance. The apparent expansion of the gray matter surrounding the sulci was due to an infiltration of the pial prolongations with cells, thus producing a widening of the fissure. The cells, however, were packed with material which blended with the gray matter and thus gave rise to an appearance of spurious thickening.

The ventricles were not dilated nor did any part of their lining show tumor nodules. The choroid plexuses seemed normal. No tumors were found in any part of the brain substance.

The spinal cord was clothed with the same thin yellowish brown film which covered the brain. There did not appear to be an invasion of the cord itself.

Microscopic Examination of the Brain

Under the microscope it was at once seen that the discoloration of the surface of the brain was due to the fact that the subarachnoid space was filled with cells which infiltrated every nook and cranny of that space. It was as if molten wax had been poured into the space and had then been allowed to solidify. The cells were of the same type as those which constituted the skin tumors except that the nucleus as a rule was darker and more eccentric in position. A more striking difference lay in the fact that none of the cells was pigmented. Just as the discoloration of the surface varied in intensity in different places, so the degree of infiltration of the subarachnoid space varied in a corresponding manner. Although the picture in its general design was that which one associates with acute meningitis rather than cerebral tumor, yet there could not be the slightest doubt that the cells were tumor cells which, because of the special anatomic arrangements, spread out in a diffuse manner instead of forming a neoplasm.

In the cerebral cortex there were numerous masses of cells which at first sight appeared to be completely separated from those in the subarachnoid space and which were so markedly perivascular in their grouping as to suggest that they were derived from the vascular endothelium (Figs. 1 and 2). In occasional fortunate sections, however, it was clearly seen that these perivascular sleeves accompanied the vessels passing from the meninges into the cortex and that the cells were directly continuous with those in the subarachnoid space. That is to say, the infiltration of tumor cells had flowed along the perivascular prolongations of the subarachnoid space for a variable distance into the brain substance (Figs. 3 and 4). A remarkable feature of these perivascular masses was that the cells in many cases were loaded with yellow pigment, although those in the adjoining subarachnoid space were completely free from pigment.

This may possibly be accounted for by the more advanced age of the deeper cells.

The walls of both lateral ventricles (thalamus and caudate nucleus) were carefully examined but no tumor cells or any implants on the ependyma could be found. A number of large cells were observed in the choroid plexuses of the lateral ventricles. It was not possible, however, to decide whether these should be regarded as tumor cells.

Tumor cells were present in the subarachnoid space and in the subjacent brain tissue of the midbrain, pons and medulla. The cranial nerves issuing from these parts of the brain carried with them a sheath of tumor cells which penetrated between the main bundles of fibers.

The meninges covering the cerebellum were infiltrated with tumor cells but none was observed in the cerebellar cortex. The same was true for the spinal cord which was surrounded by a sheath of cells particularly thick in the anterior and posterior fissures. The substance of the cord and the central canal were uninvolved.

Summing up the necropsy findings, we may say that there were collections of tumor cells of the melanoma type in the skin, in the kidney and in the subarachnoid space of the brain and spinal cord; that these cells were continued into the perivascular and perineural continuations of the subarachnoid space; and that the cells in these latter situations were pigmented, whereas those in the subarachnoid space were not.

Discussion

It has already been pointed out that diffuse infiltration of the meninges, the condition commonly known as diffuse sarcomatosis of the meninges, may arise in one of three ways. It may be primary in the meninges, it may be primary in the brain and secondary in the meninges and finally it may be primary elsewhere and secondary in the meninges with or without involvement of the brain. In the first instance the tumor is a melanoma, in the second it may be a glioma or a melanoma and in the third it may be a carcinoma, a sarcoma or a melanoma.

True primary sarcomatosis (melanosis) of the meninges is probably a condition of great rarity. The term, however, must not be used in a loose manner. In a recent communication on "Primary

Sarcomatosis of the Leptomeninges," Ford and Firor [3] give details of four cases. In the first of these there was a tumor in the right hippocampus, in the second there was a tumor in the fourth ventricle and in the remaining two no necropsy was performed. Under those circumstances one is hardly justified in speaking of the meningeal condition as being primary.

Even in the absence of a tumor in the brain, caution must be observed before concluding that the condition originated in the meninges. The eyes must first be examined for the possible presence of a melanoma. As Bland-Sutton [4] points out, while melanoma of the choroid usually occurs as a discrete tumor, it may occasionally assume the form of a diffuse flat infiltration the quiet growth of which makes detection difficult. Some cases of melanosis apparently primary in the meninges may thus in reality be secondary to unrecognized tumors in the eye.

When all these possibilities have been excluded there still remains a small group of cases in which the condition appears to have originated in the meninges. The pia, especially that portion which covers the anterior surface of the medulla, contains many pigmented cells. In the negro, indeed, this part of the brain may present a darkening which can be recognized by the naked eye. From these cells a primary melanoma may take its origin.

While the possibility of a primary meningeal origin of these tumors must be admitted, it should not yet be regarded as proved. The anatomic findings in the supposed primary cases are exactly duplicated in cases which are undoubtedly secondary. This is well seen in a remarkable case recently reported by Weller [5] in which diffuse melanotic involvement of the meninges was associated with a recurrent melanosarcoma of the skin. In this case the body was simply riddled with metastases, nodules being found in the lungs, spleen, liver, kidneys, adrenals, bladder, prostate, testes, pancreas, intestinal wall and all the lymph glands, while the myocardium was studded with melanotic nodules. Melanomas were present in the floor of the fourth ventricle and in the choroid plexus so that either of these sites, both of which are exposed to the stream of cerebrospinal fluid, may have served as the invading points for the meninges. Our own case serves as an intermediate link between Weller's case and those of the supposed primary melanoses of the meninges.

There still remains to be considered a group of cases in which

melanosis of the meninges is associated with melanomas of the skin, but in which the two conditions are supposed to be quite independent. Rokitansky,[6] Oberndorfer,[7] Grahl[8] and Maclachlan[9] have described cases of extensive pigmentation of the brain associated with pigmented nevi in the skin. The cerebral pigmentation was confined to the meninges, the surface of the brain and the lining of the ventricles. The pigmented cells in the cerebral cortex showed a marked perivascular arrangement. None of these authors considers that the pigmentation of the brain was in any way secondary to the pigmented nevi in the skin. Berblinger[10] in a very careful study comes to the same conclusion. The title of his paper, "Multiple Melanomata of the Skin with Neurofibromatosis of the Cutaneous Nerves, Melanotic Tumor of the Cerebrum, Glioma of the Pons, Sarcomatosis of the Meninges, and High Grade Congenital Hydrocephalus in a Child of Nine Months," summarizes the pathologic lesions found. He considers that two separate processes were at work: (1) melanomas of the skin and (2) melanoma of the brain which was secondary to a primary melanosis of the membranes. The perivascular arrangement of the tumor cells in the cerebral cortex was identical with that of our own case.

The principal reason why Berblinger refuses to admit that the tumor in the meninges can have any relation to those in the skin is that no other secondary growths were found in the rest of the body. To this, two points may be raised in answer. First, Ribbert has shown that melanomas may metastasize while as yet the primary nodule shows no sign of malignancy. And second, if in our own case the nodule in the kidney which was undoubtedly metastatic in nature had either been overlooked or had not been present, the same argument could have been urged against a causal relationship between the meningeal and skin lesions; whereas there cannot be the slightest doubt that the one was directly dependent on the other. The matter is not susceptible of direct proof, but it appears much more reasonable to suppose that with occasional connecting links such as the metastatic nodule in the kidney the two conditions are related and not separate and distinct.

The second group of cases is that in which a primary tumor of the brain is associated with secondary diffuse involvement of the meninges. There is no occasion to say much on this subject. In all the cases we have found described in the literature, the primary tumor

has been situated in some part of the brain with direct access to the cerebrospinal fluid, usually in the wall of the third ventricle or the floor of the fourth ventricle. The tumor erupts into the subarachnoid space just as a carcinoma of the stomach or the ovary may erupt into the peritoneal cavity and scatter its cells broadcast throughout that sac. Small implantation growths may occur on the lining of the ventricles, as in a case described by Stanley Barnes,[11] in which a tumor of the frontal region had ruptured into the lateral ventricle and was associated with several small soft masses scattered throughout the lateral, third and fourth ventricles and the aqueduct of Sylvius.

With regard to those cases of evident metastases to the meninges from primary tumors situated outside the brain or spinal cord, sufficient reference has already been made in the opening pages of this paper. Although carcinoma, sarcoma and melanoma are the principal tumors recorded in the literature, there appears to be no reason why any type of malignant new growth should not behave in this manner.

Cells in the cerebrospinal fluid. In many cases of diffuse tumors of the meninges, including our own, peculiar cells have been found in the cerebrospinal fluid. These cells, sometimes of great size, may be multinucleated and even show mitotic figures. Usually they are taken for large lymphocytes or an endothelial type of cell. The cytoplasm is clear, often vacuolated and the nuclei are large and strongly basophilic. Some excellent figures of these cells appear in an article by Chatelin [12] in the Nelson Loose-Leaf Medicine. These cells must be regarded as true tumor cells. The fact that in melanomas they do not contain pigment must not be taken as an argument against their neoplastic nature, for we have already seen that the tumor cells in the subarachnoid space may be quite free from pigment as in our own case.

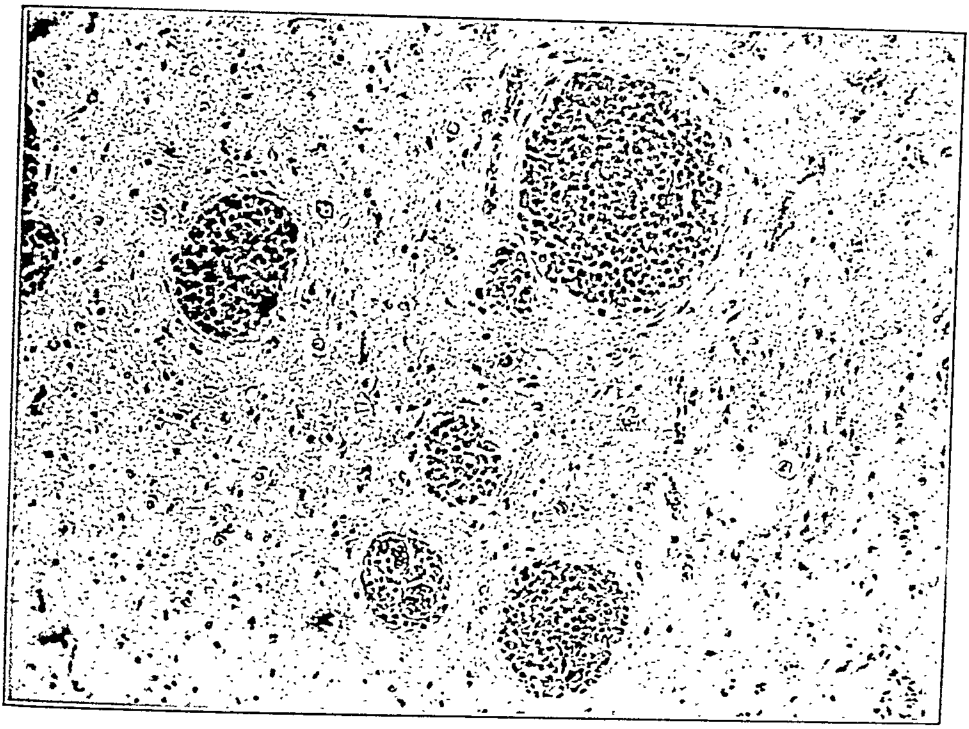
In conclusion, we may inquire what is the mechanism by which the tumor cells are distributed in such a case as that which has been described in this paper. It must at once be apparent that the cerebrospinal fluid is vitally concerned with this spread. Not only is the subarachnoid space both of the brain and of the spinal cord filled with tumor cells; not only are the primary tumors always situated in parts of the brain or cord readily accessible to the fluid; not only do the implantation growths occur solely in those parts which are

bathed by the fluid; but even the uttermost bounds of the subarachnoid space, even its farthest extensions along the vessels which penetrate into the brain and along the nerves which leave it, are permeated by the tumor cells. This permeation appears to be of a passive rather than an active character, for we could find little or no evidence of actual invasion of the brain substance. It is as if the tumor cells had been poured into the subarachnoid space as into a mould and had set there in the form of a jelly.

Both the gross and the microscopic appearance remind one of the picture of meningeal inflammation. Oppenheim[13] indeed speaks of a diffuse meningeal process rather than a true tumor formation. Lewkowicz[14] of Warsaw has pointed out in a recent paper that the prevailing views regarding meningeal infection fail to satisfy the needs of the case and that it is highly probable that instead of the infection commencing in the meninges it really originates in the choroid plexus as an ependymitis. The inflammatory cells, he believes, are carried by the flow of cerebrospinal fluid from the ventricles outward to the subarachnoid space and upward to that part of it in relation to the vertex of the brain. Some such mechanism may also be at work in cases of diffuse sarcomatosis of the meninges. From a focus in the choroid plexus, or in the case of a primary brain tumor in the cerebral substance, the tumor cells are carried outward by the flow of the cerebrospinal fluid and passively fill the subarachnoid space and all its ramifications.

Summary

Diffuse infiltration of the meninges with tumor cells, commonly known as diffuse sarcomatosis of the meninges, is in the great majority of cases a secondary condition. While the possibility of a primary meningeal origin must be admitted, this possibility is becoming open to doubt. The mechanism of the meningeal involvement is invasion of the cerebrospinal fluid with tumor cells from some focus standing in intimate relation to the fluid, such as the choroid plexus or the wall of the ventricles. A clinical diagnosis of the condition may be made from the discovery in the spinal fluid of large pale cells in considerable numbers.



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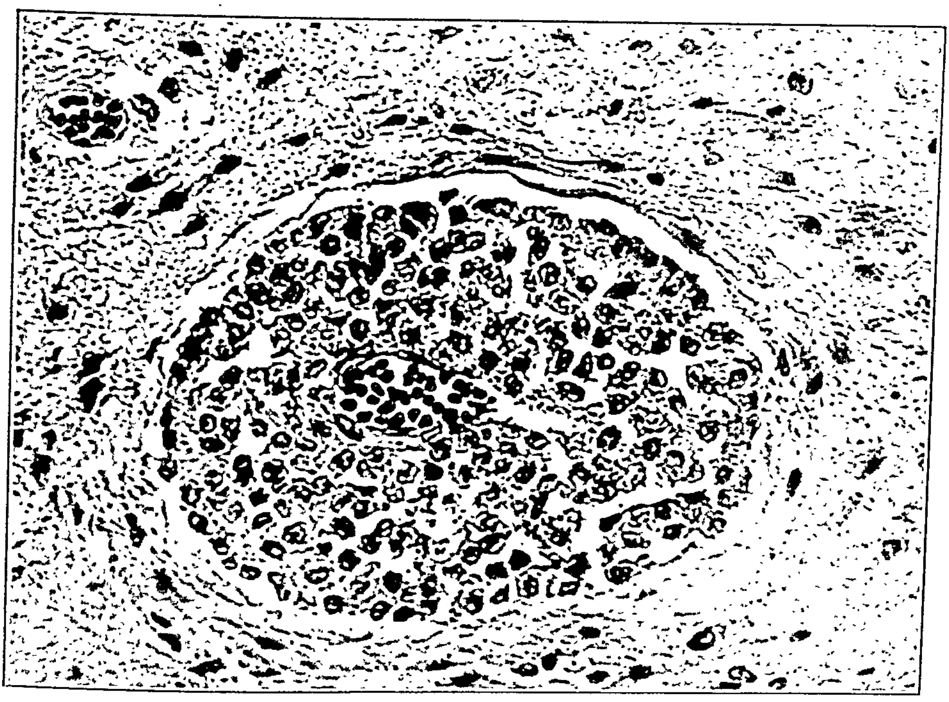

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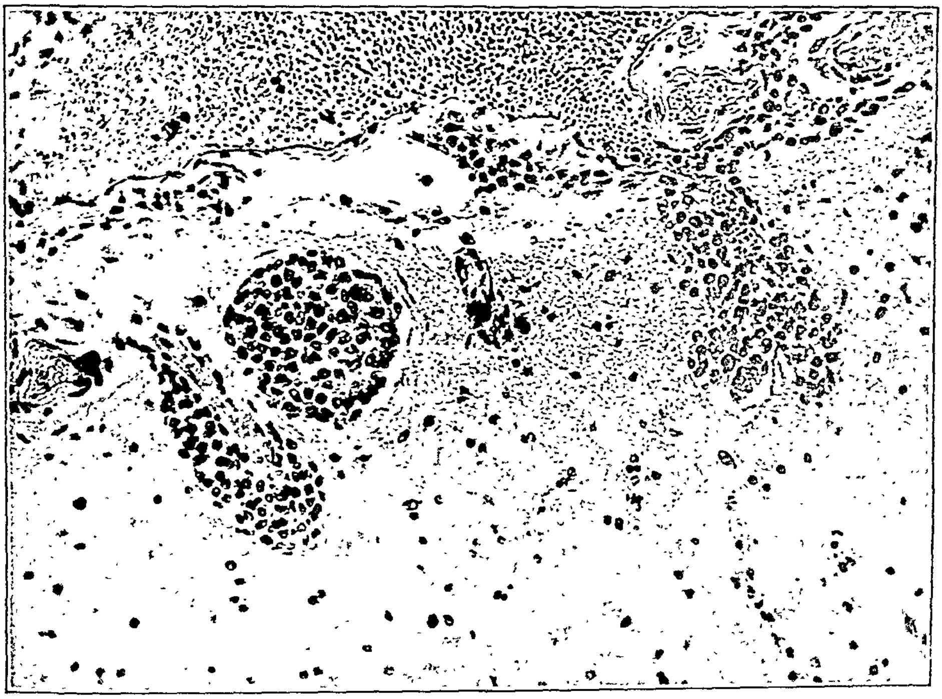

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DESCRIPTION OF PLATES XCIV–XCV

FIG. 1. Groups of tumor cells scattered throughout the cerebral cortex and apparently not connected with the surface.

FIG. 2. Mass of tumor cells grouped around a small vessel.

FIG. 3. Showing the true method of formation of the tumor masses by extension of the cells along the perivascular prolongations of the subarachnoid space into the substance of the brain.

FIG. 4. The meninges in the fissures are infiltrated with tumor cells.

SPLENOMEGALY (TYPE GAUCHER) AND LIPOID-HISTIOCYTOSIS (TYPE NIEMANN) *

WILLIAM BLOOM

(From the Snydacker Fund of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research, Chicago)

Since the description by Gaucher [1] in 1882 of an unusual symptom complex associated with large cell splenomegaly, numerous reports of supposedly similar cases have appeared. The diagnosis of a few of these has occasioned much controversy and the nature of some of them has not yet been settled. According to Pick [2] there are in the literature thirty-two well authenticated cases of Gaucher's disease; eighteen of these were studied at necropsy and in fourteen the surgical removal of the spleen yielded material for study. We have had the opportunity of studying material from two cases of Gaucher's disease and from three cases of a condition which until recently has been considered by some as Gaucher's disease. In a very recent exhaustive study of splenomegaly of the Gaucher type by Epstein [3] several cases of the condition which is certainly not Gaucher's disease are still counted as examples of it. We are, therefore, making this report to emphasize the striking differences between Gaucher's disease and this other condition and to attempt to throw some light on the nature of the latter.

GAUCHER'S DISEASE

The clinical and anatomic features of Gaucher's disease are so constant and have been recorded so frequently in recent articles that it is unnecessary to consider them in any detail here. Clinically it is characterized by its marked chronicity, leucopenia, mild secondary anemia, slight tendency towards bleeding, a peculiar brown-yellow pigmentation of the skin, splenomegaly and cirrhosis of the liver without ascites. Anatomically one finds in the liver, spleen, lymph nodes and bone marrow peculiar large pale cells which have been ascribed to various sources. Gaucher described the disease as an "épithéliome primitif de la rate." Cornil [4] thought the large

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cells in this case to be derived from reticulum cells. In the opinion of various authors since then, these cells arise from lymphocytes, endothelium or reticulum cells alone, or from both reticulum cells and endothelium. The careful and extensive studies on this subject made by Risel,[5] Mandlebaum[6] and Pick indicate that the Gaucher cells are reticulum cells and possibly certain endothelial cells which have stored large amounts of a substance which, according to the chemical investigation of Epstein,[7] seems to be closely allied to cerebrin.

The Gaucher cells with certain stains, especially with Mallory's aniline blue connective tissue stain, show a distinct longitudinal striation of the cytoplasm. They frequently give a positive iron reaction with the Turnbull blue method. None of the lipoid staining reactions is typical. After mordanting in potassium bichromate there is a very pale yellow or blue-staining of these cells with Sudan III or Nile blue. Anisotropic bodies are seen but very rarely in them.

The morphologic and histochemical reactions mentioned in the above paragraph are so constant in a very large percentage of the cases reported as examples of Gaucher's disease that they have been suggested as the criteria to be applied to all cases resembling this disease. They are evident in characteristic fashion in two cases reported herewith.

Report of Two Cases of Gaucher's Disease

Case I. R. M., No. 61854, a Jewish boy aged 6 years was admitted January 13, 1925, to the surgical service of Dr. A. A. Strauss with the complaints of enlarged abdomen, vomiting and nose bleeds. Family history and past history were negative. Three older brothers were quite healthy.

The parents of the child "always knew that the abdomen was enlarged and paid no attention to it." A physician, who was called to see the child because of a hemorrhage from the nose, found an enlarged spleen. Nose bleeds had occurred every two or three weeks for the past three years. During the month before admission the child received Fowler's solution. In this period there was slight bleeding from the nose every day. The appetite had always been good. No blood was ever seen in the stools and no petechiae in the skin.

On examination the skin was quite pale. The sclerae had a slight bluish tinge. The examination was otherwise negative except for the abdomen. This was enormously enlarged, soft and symmetrical. The spleen extended to the iliac crest in the left axillary line and below the umbilicus in the midline. It was smooth and not tender; the notch was felt near the umbilicus. The liver extended one hand's breadth beneath the costal margin and was smooth, firm and painless. There was no evidence of fluid in the abdominal cavity.

The blood on admission, Jan. 14, 1925, contained hemoglobin 53 per cent; red blood cells 2,810,000 and white blood cells 1,550 per c.mm.; neutrophiles 34 per cent; small mononuclears 56 per cent; large mononuclears 4 per cent; transitionals 4 per cent; eosinophiles 1 per cent. The Wassermann reaction was negative. On January 17th, hemoglobin 50 per cent; red blood cells 2,730,000 and white blood cells 2,050 per c.mm.; fragility complete, 0.32 per cent NaCl, and partial, 0.4 per cent NaCl. The indirect van den Bergh reaction was within normal limits; the direct was negative. The urine was negative for bile pigment and urobilin.

The child was given a transfusion of 200 c.c. of blood which was followed by splenectomy on January 20th. He improved after the operation. The blood on February 4th showed: hemoglobin 70 per cent; red blood cells 3,090,000 and white blood cells 3,400 per c.mm.; neutrophiles 42 per cent; small mononuclears 52 per cent; large mononuclears 5 per cent; eosinophiles 1 per cent.

Gross Examination: The spleen measures 19 × 12.5 × 8 cm., weighs 650 gm. and is reddish orange. The capsule is smooth and slightly thickened. On section there are many translucent grayish areas surrounded by thin red zones. They are circular, about 0.5 to 1 mm. in diameter and are probably Malpighian bodies. The rest of the pulp has a peculiar grayish orange, granular appearance. The spleen is somewhat soft. Attached to the hilum of the spleen are several medium sized lymph nodes which are soft and light gray-yellow on section. Beneath the capsules of two of these nodes are a few pin-point-sized gray-white dots. The liver is greatly enlarged, irregular in outline, uneven of surface and grayish brown. A small portion is removed at operation. There is no ascites.

Microscopic Examination: An unstained frozen section of the spleen freshly fixed in 10 per cent formalin shows most of the spleen to consist of nests of large pale cells. These are round, oval and at times fusiform in shape. The cytoplasm is composed of a hyaline homogeneous gray-white substance. In paraffin sections stained with hemalum and eosin (Fig. 1) most of the sinuses and the greater portion of the pulp are filled with nests of these large pale cells. The nuclei are fairly large, but are small when compared with the cytoplasm which in places measures 50 to 60 micra in diameter. Some of the cells contain three and four nuclei but the majority are mononucleated. The nuclei have but small amounts of chromatin material. The cytoplasm of these cells stains diffusely but only faintly with eosin. The walls of most of the sinuses are slightly thicker than normal. Some of them are lined with the large cells; in others the sinuses are completely filled with them. In some of the sinuses many red blood cells lie between the large cells and it is usually in

such places that the latter have taken up and are stained dark brown with granules of brown pigment. The few Malpighian bodies which are present are surrounded by fairly large zones of erythrocytes. A few of the follicles contain some of the large cells in the germinal centers. With Mallory's aniline blue the large cells, for the most part, are distinctly spindle-shaped; their outlines are quite indistinct and the cytoplasm contains numerous parallel wavy fibrils running in the long axis of the cells. The spaces between these fibrils are occupied by a pale blue substance. This appearance has been described in great detail by Epstein.

When stained with the Turnbull blue method for iron, most of the large cells take on a diffuse pale blue color. Under oil immersion the cytoplasm is traversed by wavy blue lines. A few of the cells are very deep blue; others have a few dark blue crystals in their cytoplasm. The diffusely dark brown, pigmented cells lose the pigment after treatment with ammonium sulphide which indicates that the pigment is a formalin precipitate. Frozen (formalin fixed) sections stained with Sudan III and counterstained with hemalum show an almost imperceptible, diffuse grayish yellow tinging of the cytoplasm of the large pale cells. In sections stained with Nile blue sulphate the large cells have a diffuse, light blue color of about the same intensity as the rest of the cells in the section. Frozen sections stained by the Lorrain Smith-Dietrich method show a light brown and in places suggestively gray cytoplasm in the large cells. The resultant color depends largely on the degree of differentiation. Certainly the reaction can hardly be considered positive. After mordanting in potassium bichromate the large cells stain a definite though very faint yellow with Sudan III and a very pale blue with Nile blue. The staining by this method is diffusely even throughout the cytoplasm. Although it is very faint, the reaction must be considered positive. In frozen sections stained with Weigert's iron hematoxylin one sees great clumps of pale-staining large cells. These stain a homogeneous bluish gray and the nuclei are darker than the cytoplasm, of which the color is approximately the same as that of the smooth muscle of the vessels. The color of the cytoplasm is somewhat darker than that of the collagen but nowhere as dark as the nuclei.

In the lymph nodes, beneath the capsule, are pale giant cells occurring singly and in small groups. They are usually placed radially

in the nodes. The cells here are quite long and with the Bielschowsky method each cell is surrounded by fine strands of reticulum fibrils. In the section of liver there is a marked increase in the periportal connective tissue. With the Mallory aniline blue stain (Fig. 2) this connective tissue is heavily infiltrated with the same large cells that are so numerous in the spleen. A few of these large cells are in the centers of the lobules. Here they have no connection with the liver cords or Kupffer cells; they appear rather to be in contact with the connective tissue about the efferent vein. This case both clinically and anatomically fulfills the criteria mentioned above for Gaucher's disease.

CASE II. Mrs. L. T., No. 13350, a Jewish woman, aged 42 years, was admitted Sept. 22, 1919, to the surgical service of Dr. A. A. Strauss. The complaints on admission were a mass in the left side of the abdomen, pain in the bladder region, weakness and slight loss of weight.

The patient had not had typhoid, malaria or pneumonia. The menstrual periods had been regular and normal until shortly before admission when they became irregular. There had been some bleeding between periods during the last two or three months. The family history was negative.

About nine years before admission, the patient first noticed in the upper left quadrant of the abdomen a small mass which grew progressively larger. There had never been any pain or discomfort connected with the mass. The pain in the bladder region had been present only for the last two or three weeks. The patient described the condition as a sensation of pressure and not a real pain. The condition simulated a desire for urination. During the last two weeks urination had been as frequent as four or five times an hour. In the patient's opinion she had lost some weight but she did not know the definite amount.

Physical examination was negative except that the spleen was palpable. It extended from the costal margin to the iliac crest and to the umbilicus in the midline and was soft and smooth.

The stools and urine contained no abnormal constituents. Blood examination on Oct. 22, 1919 revealed hemoglobin 85 to 90 per cent; red blood cells 5,600,000 and white blood cells 6,800 per c.mm.; neutrophiles 61 per cent; small mononuclears 20 per cent; large mononuclears 6 per cent; transitionals 9 per cent; eosinophiles 2 per cent; and unclassified cells 2 per cent. The Wassermann reaction was negative.

Splenectomy was performed on Oct. 30, 1919.

Gross Examination: The spleen measures 19 × 13 × 5 cm., weighs 725 gm., and is quite firm. The surface is smooth, the capsule thin, and the color through the capsule brownish red. The cut surface is uniformly brownish red, with little blood. The connective tissue is not increased and the lymphoid tissue is not visible. An accessory spleen, 1.5 cm. in diameter, is of the same appearance and is firm on section.

Microscopic Examination: The spleen is almost a replica of the one described above. The type and arrangement of the large pale cells is practically the same in both. There is, however, slightly more free splenic pulp in this case. The cells with Mallory's aniline blue stain show the typical longitudinal striations which are characteristic of Gaucher cells. The cells give a very slight pale blue stain with the Turnbull blue reaction for iron. They stain very faintly with Nile blue and Sudan III after mordanting in potassium bichromate. The Lorrain Smith-Dietrich and Fischler stains are negative, as are the Golodetsky and the iodine tests for cholesterol. The accumulation of large cells about small arterioles in the pulp is slightly more marked in this case than in Case I. In neither case is there the high grade hemosiderosis of the sinus endothelium that Pick describes. With Weigert's iron hematoxylin on frozen sections the large cells stain a pale blue-gray which is much lighter than the dark blue-black of the nuclei. This, too, is clearly a case of Gaucher's disease.

At the time of writing, five and one half years after operation, the patient is feeling unusually well. There is, as yet, no evidence of progression of the disease.

Niemann's Disease

Among the cases reported in the literature as examples of Gaucher's disease, but which on critical examination fail to fulfill the criteria of this disease, are four outlined below which are almost identical. They are neither morphologically nor chemically Gaucher's disease. The credit for the recognition of these cases as forming a distinct disease complex belongs to Pick, although Mandlebaum and Downey [8] decided that two of the cases, those of Knox, Wahl and Schmeisser,[9] should not be regarded as Gaucher's disease. The first of the four cases was described by Niemann [10] in 1914 under the title of "Ein unbekanntes Krankheitsbild." The following abstract of his report is given in some detail.

Irene D. was 17 months old on admission to the clinic. She had had difficulty in digesting her food from the second month on. The abdomen was markedly swollen because of a greatly enlarged spleen. On admission she was very poorly nourished, very apathetic and backward in development. The fontanelle was wide open. There was complete absence of any static function. The skin was flabby and very pale; the face was of a strikingly pale, brownish

color. A slight obliquity of the eyes together with several bluish spots in the skin of the back suggested the mongoloid. Breathing through the nose was markedly hindered and snorting. The abdomen measured 50 cm. in circumference. The spleen reached below the umbilicus; the liver extended to the anterior superior spine. The abdominal veins were distended and there was a low-grade ascites. There was some edema of the legs and the eyelids. The blood had a normal corpuscular and hemoglobin content. The Wassermann reaction was strongly positive. Mercury and potassium iodide were given energetically with negative results. A diarrhea set in and the child at times was slightly febrile. The child died at the age of 18 months after four weeks in the hospital.

Necropsy: There was no evidence of syphilis. The spleen was very large and fairly hard with a somewhat mottled surface. On cut surface there were yellow-white areas as large as linseeds; these were frequently confluent and between them only small strips of normal spleen. When held at a slight distance the cut surface was entirely yellowish white. The liver was likewise greatly enlarged and closely resembled the fatty liver of phosphorous poisoning. The abdominal lymph nodes were slightly swollen, of a peculiar yellow, fat-like color and rather soft in consistency. The kidneys seemed slightly fatty. The cortex of the very large adrenals was markedly yellow.

Microscopic Examination: The spleen showed but slight traces of normal splenic cells. Almost the entire organ consisted of peculiar cells. These were very large, irregular in outline and placed irregularly against each other. The cytoplasm was strikingly pale and stained red with the Pappenheim method as against the basophilia of the splenic leucocytes. There were many vacuoles in the cytoplasm of the cells. The liver was hard to differentiate from spleen. The whole organ was composed of the characteristic cells arranged in indistinct cords. There was very little connective tissue in the liver. The abdominal lymph nodes contained some large cells scattered in clumps, particularly in close relationship to the connective tissue. The cells of the adrenal could be sharply differentiated from the cells occurring in the spleen and liver in spite of the apparent similarity due to the light protoplasm. With Sudan III the cells gave no typical reaction. The color obtained was a darker and duller red than that given by fatty acids. Niemann concluded, however, "dass es sicher woll um Lipoide handeln könnte."

Niemann had never seen slides of Gaucher's disease. He believed the large cells in his case to be Gaucher cells but thought that Gaucher's disease did not occur in people under 20 years of age. He noticed, as Schultze [11] had in his lipemia case, that the sinus endothelium was uninvolved. He believed his cells, because of their close relationship to connective tissue, to be reticulum cells. He thought his case to be chemically different from those of Schultze and Gaucher, however. He suggested the name "large cell metamorphosis of glands" because the process was not limited to the spleen but involved other glandular organs.

Knox, Wahl and Schmeisser [9] have reported similar cases occurring in two sisters who were Jewish. The first child died at the age of 11 months. She had never taken her feedings well and weighed only 8½ lbs. at death. Blood examinations at various times showed a white count varying from 25,400 per c.mm. with 59 per cent polymorphonuclear and 36 per cent small mononuclear leucocytes, to 35,000 with 82 per cent small lymphocytes and 3 per cent polymorpho-

nuclear leucocytes the day before death. The child had a peculiar brownish color of the skin. The course was afebrile.

Necropsy: The liver and spleen were found to be greatly enlarged. Microscopically, large pale cells were found in the alveoli and perivascular connective tissue of the lungs, the blood sinuses of the liver, the pulp of the spleen and lymph nodes, the interlobular connective tissue of the pancreas, in some of the glomerular tufts in the kidneys, the medullae of the adrenals, the stroma and lymph follicles of the mucosa of the intestines and in the pulp of the thymus. Frozen sections showed the cells to be filled with refractile vacuoles soluble in absolute alcohol. These vacuoles were tinged light orange with Sudan III; with Nile blue they were violet-blue or pink. The cells were black by the Weigert-Pal method and many contained doubly refractile granules. "In most of the organs, especially in the liver, spleen and lymph glands, there were all transitions between cells that did not stain at all and those which contained deep orange-staining droplets with scarlet red."

Their second case was a sister of the one described above. The skin had a grayish yellow color. The superficial abdominal veins were dilated. The spleen and liver were both enlarged. There were 3,024,000 red blood cells with 11,240 white blood cells per c.mm., 60 per cent lymphocytes and 34 per cent polymorphonuclear leucocytes. Several months later the white blood count was 22,000 with 31 per cent polymorphonuclear and 67 per cent mononuclear leucocytes. The child was given radium treatment over the spleen after a biopsy on cervical and axillary lymph nodes had given a diagnosis of Gaucher's disease. Several months later the white blood count was 2,360. Examination of the eye grounds showed the cherry spot typical of amaurotic family idiocy. The child died at the age of 15 months. At necropsy the findings were quite similar to those of the preceding case. Large pale cells were noticed in the arteries and veins of the lungs, intrahepatic branches of the portal veins, the splenic arteries and capillaries of the kidneys. The young foam cells were thought closely to resemble the polyblasts of connective tissue.

Siegmund [12] reported a similar case of a girl 9 months old. The child had a large spleen and liver. The large pale cells were found in the spleen, liver, bone marrow, lymph nodes and glomeruli of the kidneys. The splenic sinus endothelium was uninvolved, although many of these large cells were in and between the sinuses. The cells stained very faintly with scarlet red and Nile blue. They were bright yellow after mordanting in potassium bichromate. They also stained with the Ciaccio, Smith-Dietrich, and Weigert's iron hematoxylin methods. Siegmund believed his case to be an early stage of Gaucher's disease.

In the cases of Niemann, of Knox, Wahl and Schmeisser and of Siegmund the patients were girls who died at the ages of 18, 11, 15 and 9 months respectively. These cases were characterized clinically by their appearance in infancy, the failure of the infants to develop, by anemia, leucocytosis, and by a large spleen and liver. In none was there evidence of diabetes or lipemia during life or at necropsy. Anatomically these cases were characterized by the presence of lipoid-containing cells in the pulp of the spleen and lymph nodes, in the thymus, mucosa of the intestine, and medulla of the adrenals, and by the presence of lipoid material in the clasmatocytes of connective tissue, in the Kupffer cells and in large cells lying free within the alveoli of the lungs. The infiltrated cells gave faint reactions with Sudan III and Nile blue but stained deeply with these dyes after mordanting in potassium bichromate and by the method of Lorrain Smith. Occasionally there were large numbers of anisotropic bodies.

Report of Three Cases of Niemann's Disease

We desire to report three similar cases. Two of these were discovered at necropsy and one was recognized during life. From the latter the spleen and a portion of the liver were removed at operation.

Case A. C. K., No. 103427, a Jewish female child 16 months old, was admitted to the service of Dr. Julius Hess, Dec. 5, 1917, with the complaints of stationary weight, diarrhea and underdevelopment. The child did not gain during the first three months. She was tried on various feedings none of which was beneficial. The child was badly constipated. She developed pneumonia. Various types of feedings were administered after this with no success. Shortly before admission a diarrhea began. The stools then contained mucus and small amounts of blood.

Physical examination showed a poorly nourished, anemic girl baby with a red macular rash on the left side of the face and thorax. The fontanelle was still open and depressed. The throat was slightly injected. A few cervical glands were palpable. The lungs were resonant and there were a few râles at the bases. The abdomen was distended and tense. The liver edge was sharp and firm and was palpated four fingers' breadth below the costal margin. The spleen was palpated two fingers below the costal margin; the border was hard and sharp. The umbilicus was somewhat pointed. The skin lacked the normal elasticity and was somewhat bronzed. Roentgenograms of the chest revealed a marked mottling throughout both right and left lungs, which extended well out toward the periphery and was suggestive of tuberculosis. The urine was normal. The Wassermann reaction was negative. The blood examinations showed on Dec. 5, 1917: hemoglobin 90 per cent; red blood cells 4,860,000 and white blood cells 9,100 per c.mm.; neutrophiles 28 per cent; small mononuclears 54 per cent; large mononuclears 10 per cent; transitionals 3 per cent; eosinophiles 3 per cent; basophiles 2 per cent. On December 12th the blood had: red blood cells 4,700,000 and white blood cells 19,600 per c.mm.; neutrophiles 50 per cent; small mononuclears 29 per cent; large mononuclears 15 per cent; transitionals 5 per cent; eosinophiles 2 per cent; basophiles 1 per cent. On December 16th there were red blood cells 4,900,000 and white blood cells 36,000 per c.mm.; neutrophiles 53 per cent; small mononuclears 34 per cent; large mononuclears 8 per cent; transitionals 4 per cent; eosinophiles 1 per cent; hemoglobin 70 per cent. The von Pirquet test was negative.

Necropsy: A necropsy was done by Dr. Perlstein with the following findings.

External Examination: The body is that of a markedly emaciated female infant. The anterior fontanelle is open and measures 2.5 × 2.5 cm. The posterior fontanelle is closed. The pupils are normal. The corneae are cloudy. The very large abdomen is tense and the umbilicus is protuberant. The spleen can be felt to extend down to the crest of the ilium. The liver can be felt 6 cm. below the lower

end of the sternum in the sternal line. There are numerous bluish brown discolorations of the skin, which are present only on the right side of the thorax and abdomen. They are well-defined, round in shape and measure up to 0.4 cm. in diameter. The glands in the axilla and groin can be felt.

Internal Examination: Very little subcutaneous fat is present. When the peritoneum is incised some clear yellowish fluid escapes. The omentum is dotted with numerous small white nodules which measure up to 2 mm. in diameter. They have some similarity to tubercles. Hardly any fat is left in the omentum. The spleen and liver are very large and occupy the greater part of the abdominal cavity. In the dependent portions of the thorax there is also a considerable quantity of fluid.

Heart: The heart is of normal size. The valves are normal. The endocardium is smooth and glistening and the myocardium has the normal color.

Lungs: The lungs are large, dark red in color, congested and edematous. On section a blood-tinged frothy serum escapes. Nowhere can induration be felt. The peribronchial lymph nodes are not enlarged.

Thymus: The thymus weighs 8 gm. It is pale and appears edematous.

Liver: The liver measures 20 × 12 × 6 cm. and weighs 580 gm. Its surface is smooth but slightly uneven. It is pale yellow to salmon-pink in color. On section it is of the same color and fatty to touch. Throughout the organ the central veins stand out as small minute dark red dots in the yellowish pink ground substance. The gall bladder and bile passages are normal.

Spleen: The spleen measures 12.5 × 7.5 × 3.5 cm. and weighs 190 gm. The surface is smooth, the capsule tense and the organ firm. It is pale pink with some irregular areas of a darker red scattered through it, giving it a mottled appearance. Its edges are rounded. On section it is pale pink and scattered through it are some irregular dark red areas which have the color of normal splenic tissue. The splenic veins are of normal appearance.

Kidneys: The left kidney measures 7.5 × 3.5 × 1.5 cm. and weighs 30 gm. The right kidney measures 7 × 4 × 2 cm. and weighs 32 gm. The capsules of both strip without difficulty. On section the kidneys are paler than normal. The anatomic landmarks

are well defined although the tissue is somewhat cloudy. The cortex in its thickest portion measures 0.6 cm.

Adrenals: The right adrenal measures 4 × 2.4 × 1 cm. and weighs 5 to 6 gm. Its cortex is prominent and thicker than normal. The left adrenal measures 3.8 × 3 × 1 cm. and weighs 8 gm. It is of the same appearance as the right adrenal.

Pancreas: The pancreas weighs 18 gm.; it is pale, softer than normal and feels fatty.

Intestines: The intestines are pale. In the lower portion of the ileum a number of enlarged Peyer's patches are seen. They are pinkish red and slightly raised over the surface of the grayish mucosa. They are 2 to 3 cm. long and about 1 cm. wide. A few of these patches are seen farther up in the ileum, but none is seen in the jejunum. The mucosa of the large intestine is studded with enlarged lymph follicles which are especially numerous in the proximal portion and which project from the mucosa as small nodules. They measure up to a few millimeters in diameter.

The *lymph nodes* of the mesentery are markedly increased in size and number. They measure up to 1.5 cm. in diameter and are pale and of a grayish color on section. Several large lymph nodes are present in the meso-appendix. The retro-peritoneal lymph nodes are increased in size.

Microscopic Examination: All sections were made by the writer from tissues fixed in formalin and preserved in 80 per cent alcohol since 1917.

Heart: The heart muscle fibers in places are slightly vacuolated and are suggestively embryonal in character in that the fibrils are grouped around the periphery of the sheath and the centers of the fibers are vacuolated.

Thymus: Most of the organ has been replaced by pale cells, the process being much more marked in the cortex than in the medulla. The comparatively few collections of lymphoid cells are, for the most part, rather compactly arranged around the Hassall's bodies. In some places the pale cells come almost up to the Hassall's bodies (Fig. 3). With Mallory's aniline blue the cells stain a delicate vacuolated blue; some are multinucleated. Thin strips of reticulum separate the pale cells into large groups. The reticulum, however, does not always extend around the individual cells. In the areolar tissue between the lobules, the large pale cells, which here stain with

a little more red than within the lobules, are in closer relation to the reticulum and collagen fibrils.

Lungs: Sections taken through various parts of the lungs show most of the alveoli to be filled with coagulated albuminous material. Polymorphonuclear leucocytes are present in a few of the alveoli. Many of the alveolar spaces are occupied by large foam cells which are frequently multinucleated (Fig. 4). Some of these cells are much larger than the usual dust cells. The capillaries in the alveolar wall not infrequently contain similar cells, which usually are smaller than the large cells in the alveolar spaces and in the branches of the pulmonary artery. In another section there is a small lymphoid area consisting of a few lymphoid cells, typical lymph sinuses and large numbers of pale vacuolated cells apparently identical with those in the mesenteric lymph nodes and the spleen. These cells do not radiate in strands along the contiguous alveolar septa. One septum, however, is quite rich in these cells, and with the aniline blue stain one gets the impression that they may be contained within lymphatics. In the branches of the pulmonary artery there are quite a few of the large cells, sometimes as many as three and four in a single high power field. The large cells are also very frequent in the connective tissue about the arteries and bronchi.

Liver: In the hemalum and eosin preparation the liver cells stain lightly and are interspersed with the pale-staining cells described above. There is a slight increase in the periportal tissue, and not infrequently there are small groups of lymphocytes and polymorphonuclear leucocytes in the periportal areas. With the low power it is very difficult to delineate the cords of liver cells. With the high dry objective, however, the liver cells are finely stippled, pink-staining cells with round, rather darkly staining nuclei. Between the liver cords pale-staining cells lie apparently in the sinusoids. Few sinusoids contain blood and practically no normal Kupffer cells can be recognized. The nuclei of the pale cells vary much in shape; some are round, others are irregular in shape, and still others are markedly elongated. The cytoplasm of these cells is distinctly vacuolar in character (Fig. 5). Occasionally long spindle-shaped nuclei line the spaces containing these large cells. With the aniline blue stain the distinction between the liver cells and pale cells is well brought out. The liver cells stain a coarsely granular, orange-red; the nuclei vary from yellow to orange-brown, while the pale

cells are light blue. The reticulum and collagen are unusually well demonstrated. Each liver cord is bounded by a thin strip of dark blue reticulum. The pale cells with their vacuolated cytoplasm lie, for the most part, between the liver cell cords and the covering reticulum. In the periportal areas there is a marked increase in reticulum. In these areas the pale cells predominate, sometimes almost to the entire exclusion of liver cells in large portions of a high power field. With this stain none of the cells has the longitudinal striation characteristic of Gaucher cells.

Spleen: With hemalum and eosin the organ bears very little resemblance to a normal spleen; it is made up practically entirely of groups of pale cells (Fig. 6). A few strands of thick, deeply red-staining fibrous tissue run through the organ and the capsule is normal in appearance. With some difficulty the sinuses free of foam cells can be picked out. With Mallory's aniline blue stain, however, the sinuses stand out quite prominent with the low power as thin, double-walled, purplish blue cords against the fairly homogeneous bluish gray background composed of closely packed foam cells (Fig. 7). With the higher power the sinus endothelium is low and flat, and an occasional sinus lumen contains a few comparatively small foam cells; sometimes, however, a foam cell as large as those in the pulp is seen. In places, at dilatations of the sinuses, the pulp appears to be pressing into the lumen of the sinus. The sinuses are compressed by the foam cells outside them. The Malpighian bodies consist almost entirely of foam cells. The latter are closely packed about the vessels. In tracing the trabeculae one finds the blue-staining collagen in places suddenly spreading out in finger-like processes into the reticulum fibrils of the pulp. These fibrils are separated by small numbers of foam cells. The foam cells proper are usually round, oval or slightly polygonal in shape and vary in size from 20 to 60 micra. The nuclei are comparatively small; occasionally a cell is multinucleated. The cytoplasm is very finely vacuolated; but in some cells the vacuoles have coalesced, so that the whole cytoplasm may consist of two or three large vacuoles.

Pancreas: The pancreas itself presents nothing abnormal and is surrounded by fetal fat. A few pale cells with various degrees of vacuolization lie in the connective tissue about the organ. This is particularly well marked around a comparatively large vein which runs through the section.

Mesenteric Lymph Node: A preparation stained with Mallory's eosin-methylene blue or hemalum and eosin shows the node to be composed of diffusely distributed large cells with pale, faintly vacuolated cytoplasm and relatively small nuclei (Fig. 8). The cells are confined almost entirely to the pulp. Large pale-staining cells are more numerous in the centers than in the peripheries of the few typical follicles present. The lymph sinuses are not particularly prominent and they do not contain any of the large pale cells. The loose areolar tissue surrounding the lymph nodes contains many of the pale cells which are smaller than those in the lymph node proper. The nucleus is eccentric and small, and apparently all gradations can be traced between small, oval or spindle-shaped cells with slightly elongated nuclei, through cells of the same shape containing finely vacuolated pale-staining cytoplasm, up to the typical pale cell occurring in the pulp of the spleen. The earliest stage of this process involves what are probably young reticulum cells. Section stained with aniline blue shows the reticulum fibrils very nicely. Most of the lymph sinuses are free from large cells and the pulp is the portion apparently most affected. With this stain the cytoplasm of the pale cells is quite prominent, because of a very definite light blue vacuolization. Nothing suggestive of the typical longitudinal striation of the Gaucher cell is seen. In the loose areolar tissue about the node the pale cells are much more prominent with this stain than with hemalum and eosin and the transition stages are even more plainly marked by this method.

Kidney: The tubular epithelium in places is quite thin and finely vacuolated. Some of the glomeruli contain large, pale, finely vacuolated cells in their tufts (Fig. 9). The very small branches of the renal artery contain these cells, which are frequently found in the arterioles up to the Malpighian bodies.

Adrenal: The cells of the cortex are slightly vacuolated. The medulla is greatly thickened with large numbers of foam cells. These are most numerous at the cortico-medullary junction. Many of them, however, lie within capillaries. With Mallory's aniline blue stain the blue foam cells stand out quite sharp against the red of the cortical and medullary cells.

Small Intestine: Scattered through the lower layers of the mucosa are a few large pale-staining cells; these are not prominent and are usually spheroidal. The nucleus contains a very darkly staining

nucleolus and a very indistinct chromatin network. Heidenhain's iron hematoxylin shows the vacuolated appearance of the large cells very beautifully. In some of the cells the vacuoles are quite small and in others they are confluent and form large droplets. The large cells are quite abundant, in one section, in the lymph follicles of the mucosa. There are quite a few of the large cells in the fibrous portion of the submucosa.

With special staining methods for lipoids the pale cells scattered throughout the various organs in this case give practically uniform reactions. With Sudan III the cells take on a yellowish brown color. When these sections are counterstained with hemalum the cytoplasm takes up some of the hemalum so that the appearance is a grayish brown; with Nile blue sulphate the foam cells stain a very delicate lavender. After mordanting in potassium bichromate and staining with Sudan III the large cells contain variously sized granules of highly refractile bright yellow material. In similarly prepared sections stained with Nile blue sulphate the cytoplasm of the large cells is filled with lavender granules. In frozen sections stained with Weigert's iron hematoxylin the cytoplasm of the large cells is composed of dark bluish black granules. In places the cells have taken on so much of the stain that the nuclei are completely obscured. In the lung the large cells also take the Weigert's iron hematoxylin and large black cells are seen in the branches of the pulmonary artery. With this stain, too, the large cells in the medullae of the adrenals stand out unusually prominently. The large cells in this case do not react positively to tests for iron. In frozen sections their cytoplasm is light blue with hemalum and purplish red with alum carmine. The basophilia of the cytoplasm disappears after treatment with absolute alcohol.

Case B. No. 62969, a Jewish female infant 14 months old was admitted to the pediatric service of Dr. I. A. Abt on Feb. 25, 1925, and discharged March 19th.

The infant's development had been rather slow. She held her head up at 4 months. She could not sit alone nor stand at the time of admission. The first tooth had appeared at 9 months. She had been breast fed for 7 months and then various feeding mixtures were tried. Orange juice and cod liver oil had been administered in large amounts. The family history was unimportant.

At the age of 7 months the mother noted that the infant would take no other feeding than milk and also that it made no effort to sit up or move. A physician was consulted who found nothing wrong with the baby. She continued a subnormal development and did not smile or seem as active as she should be. When

the baby was about 8 months old the mother noted an enlargement of the abdomen which a physician said was due to an enlarged liver and spleen. At the age of 12 months the adenoids were removed. The baby had been keeping her mouth open for various lengths of time. She nursed readily but vomited the 6 o'clock feeding occasionally. The bowel movements were normal. She had lost 1 lb. in the past month.

Physical examination revealed a markedly anemic and deformed child who was quiet during the entire examination. The fontanelle was nearly closed. The pupils did not react to light. The conjunctivae were subicteric. The upper lids were swollen and there was a fine nystagmus of both eyes. Breathing was obstructed, and the tonsils were enlarged. The lips were very pale. The cervical lymph nodes were palpable on both sides. The lungs were normal. The lower thoracic spine was kyphotic and the ribs flared out. The abdomen was quite prominent and distended. The liver was palpable as far down as the anterior superior spine. The spleen extended on the left side to the anterior superior spine; the notch was palpable at the level of the umbilicus. No fluid wave was made out in the abdomen. The legs were quite small and there was a bilateral talipes. The left hand had only the distal phalanges of four fingers. The epiphyses of all the bones were enlarged. The knee and ankle reflexes were not obtainable.

Splenic puncture was done on March 3rd. The material obtained was a light, yellowish pink bit of tissue measuring $2 \times 2 \times 1$ mm. It was touched to several cover slips, a few cells were teased in salt solution and the remainder of the tissue dropped into 10 per cent formalin. Examination of the material in salt solution showed many red and white blood cells amongst which were scattered a few very large cells measuring 30 to 40 micra in diameter. They were opaque yellowish gray, and with higher magnification were irregular in outline as though the cytoplasm was composed of many variously sized bubbles. When Sudan III was applied to these cells they became orange-gray in color. The bit of tissue placed in formalin was imbedded in paraffin and serial sections cut. These were mordanted in 1 per cent chromic acid and stained with Mallory's aniline blue. The greater part of the section was made up of very large, vacuolated, faintly blue-staining cells which surrounded the few sinuses in the section. The picture so closely resembled that in the sections of the spleen from Case A that the diagnosis was ventured that the case under observation was very probably quite similar to that one.

Laboratory Findings: Hemolysis was complete with 0.25 per cent NaCl, and partial with 0.4 per cent. The urine was normal. Blood examinations: March 1st: red blood cells 4,200,000 and white blood cells 23,400 per c.mm.; neutrophiles 45 per cent; small mononuclears 37 per cent; large mononuclears 8 per cent; transitionals 7 per cent; myelocytes 3 per cent. March 13th: hemoglobin 45 per cent; red blood cells 3,560,000 per c.mm.; neutrophiles 40 per cent; small mononuclears 42 per cent; large mononuclears 8 per cent; transitionals 7 per cent; myelocytes 3 per cent. The erythrocytes showed a moderate degree of anisocytosis, poikylocytosis and polychromatophilia. No nucleated red blood cells were seen. The von Pirquet test was negative. The bleeding time was 6½ minutes. Before operation the blood cholesterol by the method of Bloor was 210 mg. and the blood fatty acids by the method of Bloor were 640 mg. per 100 c.c. of serum.

On March 9, 1925, splenectomy was performed.

Gross Examination: A piece of liver excised at the time of operating is yellowish pink and measures 4 × 4 × 3 mm. The spleen measures 13 × 8 × 4 cm. and weighs 268 gm. One small notch is 1.5 cm. deep. One aberrant vessel enters the spleen 3 cm. from the hilum. The spleen exteriorly is a light salmon color. The capsule is rather thin and contains frequent, slightly depressed pin-point-sized areas. On section the spleen is salmon-yellow. Scattered through the pulp are small, slightly translucent, grayish pink areas usually less than 1 mm. in diameter. These usually have a thin layer of red tissue surrounding them. On holding the organ at various angles one sees numerous, very small, glistening particles making up the pulp; these suggest fine flakes of mica in appearance. The pulp consistency is resilient, slightly doughy and poor in connective tissue. The trabeculae are not prominent.

Microscopic Examination: The spleen is practically a duplicate of that of Case A. When stained with hemalum and eosin the great mass of the organ is composed of pale-staining cells with small nuclei and a vacuolated pink cytoplasm. The cells are confined almost entirely to the pulp, usually in the vicinity of small arterioles. The lining of the sinusoids is apparently uninvolved in the process, although there are occasional foam cells in the lumen of the sinuses. The reticulum in the centers of the Malpighian bodies is quite prominent and there are a few foam cells in the germinal centers. Hematopoiesis occurs in some of the sinuses. With Mallory's aniline blue stain the distribution of the foam cells is even more characteristic. They have a distinctly blue, vacuolated cytoplasm. Nothing suggestive of the longitudinal striation of the Gaucher cell is seen.

The liver at first glance shows quite an atypical structure. With hemalum and eosin a slight tendency of the cells to group themselves into two classes can be made out. One group stains lighter than the other with the eosin. With Mallory's aniline blue stain the difference between the two types of cells is striking. One group, apparently hepatic parenchyma, stains reddish brown and is coarsely granular. The other type of cell stains distinctly blue and is practically always in places which are surrounded by fairly thin strands of blue-staining reticulum. These cells are clearly vacuolated. There is an increase in the periportal fibrous tissue and the lobules are smaller than usual.

In frozen sections after formalin fixation stained by the Lorrain

Smith-Dietrich method the pulp is yellow except for the black nuclei. Large irregular and rounded black spots corresponding in size and position to the pale cells are scattered throughout the section. Several of these are seen in the light yellow Malpighian corpuscles. By Fischler's method no soap is present. In sections fixed in Flemming's osmic acid mixture the pale cells are dark because of very many fine black granules in the cytoplasm. With hemalum and Sudan III on frozen sections the large cells are finely granular and purplish brown. With hemalum alone the granulation is dark blue. With Sudan III alone the pale cells are a light though brilliant orange; the foreign substance therefore stains with both Sudan III and hemalum. In frozen sections after extraction with acetone or absolute alcohol the pale cells are very light blue with hemalum but do not take the Sudan III. The blue is not as intense as before extraction. The sections of spleen do not react with microchemical methods for iron. With Nile blue the pale cells are a definite light lavender. Frozen, formalin fixed, section of the spleen treated with Weigert's iron hematoxylin is practically a duplicate of that seen in the spleen of Case A (Fig. 10). The large cells are almost black and stand out in prominent clumps and cords against the light bluish gray pulp. The perivascular arrangement in this section is not as prominent as it is in that of Case A, but the presence of the cells along the trabeculae is just as marked.

After mordanting in potassium bichromate and staining with Sudan III the large cells contain very many highly refractile, bright orange granules. In many of the cells the intensity of the orange varies somewhat. In sections mordanted in potassium bichromate and stained with Nile blue sulphate the large cells contain a finely granular deep lavender material. The remainder of the section has a hardly perceptible blue color.

Case C. W. K., No. A63717, a Jewish male infant, aged 7 months, was admitted to the pediatric service of Dr. I. A. Abt on Mar. 26, 1925. He died the following day.

The infant had been apparently quite normal in development until three days before admission, when he began to cough. His temperature rose as high as 104.5 and the child became semi-comatose. He lay in bed without moving, with his eyes closed and refused to take any food.

Physical examination on admission to the hospital revealed a well developed and well nourished male infant 7 months of age who was very acutely ill. The cheeks were flushed and hot and the skin was dry. The lips were cracked and dry. Respirations were rapid. There was marked nystagmus. The chest was

normally resonant throughout. A few scattered coarse râles and some areas of roughened breath sounds were detected in the chest. The spleen was enlarged, and was palpable 3 cm. below the costal arch. The liver was also palpable. There was an occasional convulsion with tonic contractions of the legs. The knee jerk was decreased on the right side; the Brudzinski was negative; Kernig's and Babinski's signs were positive on both sides; the upper extremities were spastic. Shortly after admission to the hospital the infant began having convulsions with twitching of the face and right arm. It was given chloral and morphine, which stopped the twitching. By spinal puncture markedly turbid fluid was obtained. On smear examination this showed a few red blood corpuscles and many gram-positive diplococci which were occasionally arranged in chains. The infant died that night in a convulsion. Blood examination on March 26th showed hemoglobin 65 per cent; red blood cells 3,960,000 and white blood cells 29,200 per c.mm.

Necropsy: External Examination: The body is that of a well nourished male infant, which measures 66 cm. in length. There are crusts of an eruption in the scalp above the anterior fontanelle; the latter is almost completely closed. The scalp is quite thin.

Internal Examination: Head: The bones of the skull present no external abnormalities; they are thin and can be cut easily with the saw. The dura, which is separated from the calvarium with some difficulty in the region of the sutures between the bones, is thin and non-adherent to the leptomeninges which, over the anterior portions of the cerebrum, are greenish yellow and are raised above the cerebral cortex for at least 1 cm. by a yellowish green fluid exudate. The vessels of the pia are surrounded by a similar exudate which here is slightly more abundant, so that the course of the vessels is marked out distinctly. Portions of the posterior lobes of the cerebrum are free from the exudate. The meninges at the base of the brain, especially in the interpeduncular space, are covered and thickened by an exudate similar in appearance to that described above. The brain substance proper and the ventricular system are free from involvement in the inflammatory process. The cerebrospinal fluid is slightly cloudy. Both middle ears are filled with yellowish green pus.

The hypophysis is small and covered with a slight amount of greenish fibrin. The longitudinal sinus and all of the sinuses at the base of the skull are incised and appear normal. The cribiform plate is removed and the nasal cavity examined; it is apparently normal.

The subcutaneous fat of the anterior abdominal wall is somewhat tallow-like in appearance and measures 1 cm. in thickness. The abdominal and pleural cavities are free of fluid and adhesions.

The *thymus* is quite small and white. It measures $2.5 \times 2 \times 0.5$ cm.

The *lungs* contain air throughout. They are reddish in appearance. No masses are felt within them. On section the pulmonary tissue is mottled red, gray and pink. There is no evidence of consolidation. The cut surface is rather dry and sticky.

The *heart* lies free in the pericardial cavity, which is free from fluid. The heart is of normal size. The ductus arteriosus is present, but its lumen is obliterated. The musculature of the heart is reddish brown and rather firm. The right auricle is distended with clotted blood. On incising the heart no abnormalities are noted in the valves and myocardium.

The *marrow* of the ribs is red.

In the abdominal cavity it is noted at once that the *spleen* is increased in size. The spleen measures $11 \times 5 \times 3$ cm. and weighs 62 gm. Its surface is salmon-pink in color and slightly irregular in contour. A notch extending 2 cm. into the substance of the organ is seen on the gastric surface. On section the pulp is resilient and rather firm. It is salmon-colored, tinged slightly with brown. The Malpighian bodies stand out as small translucent grayish white points surrounded by a thin zone of red which demarcates them from the lighter colored splenic pulp.

The *adrenals* are small and contain but little lipoid. The *gastro-intestinal tract* appears normal, as does the *pancreas*.

The *liver* is of normal size, light brown and the lobulation fairly distinct. The centers of the lobules have a very obvious light grayish tan color in contrast with the surrounding brown periphery.

The *mesentery* contains a few small lymph nodes. The omentum is normal.

The *kidneys* are of normal size and are light brown in color. The capsule is removed without difficulty. There are no hemorrhages in the cortex. The striations are distinct. The bladder and ureters are normal.

No *lymph nodes* are noted as being increased in size anywhere in the body.

Bacteriologic Examination: The meningeal and otitic exudates contained Type II pneumococci in pure culture.

Microscopic Examination: Thymus: There is no subdivision of the organ into cortex and medulla. It consists of lobules of thymic

tissue fairly heavily infiltrated with fibrous tissue and small amounts of adipose tissue. Scattered between the few lymphoid cells are large numbers of foam cells with decidedly pink-staining cytoplasm. The Hassall's bodies are prominent. The foam cells are distributed throughout the section and do not seem to have any particular arrangement other than a diffuse dispersement throughout the pulp, as seen by the hemalum and eosin stain. With Mallory's aniline blue stain the foam cells are seen usually to be very close to reticulum fibrils. Most of the blood vessels are free of these cells. About an occasional small vessel the foam cells are somewhat more numerous than elsewhere. The loose areolar tissue about the thymus contains a few foam cells.

Heart: The fibers are quite small, closely placed, and the striation is rather indistinct. In places the fibers are somewhat embryonal in character. The vessels in the heart muscle contain no foam cells.

Lung: Many of the alveoli are completely filled with large foam cells which are quite similar to those described in the spleen and other organs. The cells are most commonly present in alveoli adjacent to septa and beneath the pleura. Some of the alveoli contain only a few of these foam cells, which are spheroidal or polygonal in shape; those which are spheroidal are usually still attached to the walls of the alveoli. In another section of lung the large cells so completely fill the alveoli as in places to give the impression of a catarrhal pneumonia. The bronchi frequently contain large numbers of foam cells, and many are seen in the branches of the pulmonary artery.

Spleen: In the hemalum and eosin preparation, most of the organ consists of groups of large, pale-staining cells, which are 30 to 50 micra in diameter and contain a pink-staining, finely reticular cytoplasm. The type of nucleus varies. In some of the cells it is somewhat vesicular, and contains one or two small chromatin dots. In others the nuclei are quite compact and deeply stained. The cells are present diffusely throughout the tissue, but they are more numerous immediately beneath the capsule and about the trabeculae than in the remainder of the organ. The Malpighian bodies are small and are frequently surrounded by a pulp rich in red cells. Some of the germinal centers are almost completely replaced by foam cells. The same section stained with Mallory's aniline blue shows the grouping of the foam cells much more markedly than does

the routine hemalum and eosin. The venous sinuses occasionally contain a foam cell but for the most part are free. The foam cells lie in the spaces between the sinusoids and practically always surround a small vessel, as shown by the concentric arrangement of the blue-staining reticulum. The picture is quite similar to that described for certain cases of Gaucher's disease by Pick. The endothelium of the sinuses is flattened. In some areas one can trace a direct continuity between the mass of foam cells in the pulp and the lumen of a sinus. The foam cells within the sinuses of the spleen are usually smaller than those in the pulp. Occasionally one finds a comparatively large area of uninvolved pulp, but even here on close examination one can find, contiguous to loose strands of reticulum, foam cells which with the Mallory stain stand out quite sharp because of their reticulated blue cytoplasm. Study of the spleen offers fairly conclusive morphologic evidence that the foam cells in this organ arise from the cells of the reticulum, particularly about the smaller arteries. The endothelium of the sinuses is apparently uninvolved in the formation of the pale cells.

Liver: With the hemalum and eosin stain the liver has a fairly normal appearance. The cytoplasm of the liver cells stains homogeneously red with eosin and most of the Kupffer cells are inconspicuous; a few, however, are large and their cytoplasm is markedly vacuolated. Some of these cells are three and four times the size of the circumjacent liver cells. With the Mallory stain, however, one can trace an apparent gradation between the Kupffer cells with little cytoplasm through others which contain a few small vacuoles, up to the large cells which are preponderatingly vacuolated and are typical of the foam cells found throughout the other organs.

Duodenum: In the lower layers of the mucosa and submucosa are a few small foam cells embedded in fibrous tissue. Their nuclei are compact and deeply stained.

Mesenteric Lymph Node: Foam cells form fairly large clumps in the medullary portion. The peripheral lymphoid follicles occasionally contain cells with large, finely vacuolated cytoplasm. The latter are smaller than the cells seen in other organs and in the medullary portion of the node. The lymph follicles are small.

Adrenal: The cells of the zona fasiculata are somewhat vacuolated; those of the zona reticularis stain well and abut directly against a thick layer of foam cells in the medulla. The latter stand

out sharply against those of the zona reticularis, in that their axes are perpendicular to those of the cellular elements of the deepest portion of the cortex. Beneath this medullary layer of foam cells is a thin zone of engorged capillaries, which frequently contain foam cells. The original medullary cells are in general quite inconspicuous, but are more prominent about the large blood vessels. The same section stained with Mallory's aniline blue shows a similar picture, except that the foam cells stain blue by this method and stand out with even more sharpness against the medulla and cortex. Thick strands of reticulum frequently run between small groups of the foam cells and their intravascular localization is exceedingly well brought out by this method.

Kidneys: The glomeruli are small. The tubules are somewhat swollen. The vessels and glomerular tufts are free from foam cells. In a branch of the renal artery near the pelvis there are quite a few such cells. Section through the other kidney shows this to resemble the one just described, but no foam cells are found.

The subcutaneous fat is apparently normal. Striped muscle presents no abnormalities and is free from foam cells.

Brain: The meninges are greatly thickened with an exudate consisting almost entirely of polymorphonuclear, a few mononuclear white cells and fibrin. In the meningeal exudate only a few large foam cells are seen. No cells of this type are present in the vessels of the meninges. In one section the cortex is apparently normal, except for marked distention of the small vessels with blood. Another section through the brain shows in some places marked infiltration of the walls of the arteries with polymorphonuclear leucocytes, and in other areas well marked perivascular infiltration with the same cells. There is, however, in these areas no suggestion of the foam cells described in the other organs. The cerebellum shows slight infiltration of polymorphonuclear leucocytes in the meninges but no foam cells. The stroma of the choroid plexus is heavily infiltrated with cells which have a large amount of clear cytoplasm. These are the size of the foam cells present in the other tissues, but the cytoplasm has a peculiar striation rather than vacuolization and these are probably not identical with the foam cells in the rest of the body. The pars glandularis of the hypophysis is covered with a thick layer of purulent exudate similar to that observed in the meninges. The organ is hyperemic and in the center there is an ill-

defined area the size of a high power field which contains some cloudy albuminous fluid, imbedded in which are many mononuclear cells. These are larger than the monocyte of the blood and contain darkly staining, slightly vesicular nuclei and finely reticular cytoplasm. They are highly suggestive of young foam cells. The subcutaneous fibrous tissue is apparently normal.

Marrow: The bone marrow contains an occasional foam cell.

Aorta: The aorta and periaortic areolar tissue are normal.

Spleen: With Weigert's iron hematoxylin the light blue splenic pulp stands out sharply against the dark blue-black, opaque, large cells which are arranged in cords and clumps. The small arteries passing through the pulp are sheathed by a layer, two to three cells thick, of the large cells which also run in thin sheets along the connective tissue trabeculae. The cytoplasm of the deeply stained cells is filled with bluish black granules.

Liver: When stained with the same method the liver cells are diffusely bluish gray and are darker at the peripheries of the lobules than in the centers. Scattered in places throughout the organ are greatly enlarged Kupffer cells which stain almost coal black. With the higher powers they are finely granular. A careful study of the Kupffer cells throughout the organ shows most of them to stain deeply, but it is in the greatly enlarged Kupffer cells, particularly about the periphery of some of the lobules, that the black staining of these cells is unusually prominent.

Lung: The large cells in the alveoli of the lung stain intensely black with Weigert's iron hematoxylin. For the most part they are free in the air spaces, usually in those close to the larger bronchi, about the larger vessels and beneath the pleura. A few of the large, deeply stained cells are present in the capillaries and some of the smaller arteries contain similar cells.

After treatment with potassium bichromate followed by Nile blue, the liver cells are sprinkled with dark blue droplets and in places a few rods. The Kupffer cells occasionally are slightly enlarged and contain fairly large granules of light purple-staining material. The pale cells of the spleen are distinctly purple with this method.

The liver stained with Sudan III after mordanting with potassium bichromate shows many fine, yellow granules scattered throughout the liver cells. The Kupffer cells not infrequently contain small amounts of bright orange staining material.

Discussion

In the three cases just described one apparently is dealing with the same process, which is characterized anatomically by the appearance of large foam cells in the various organs examined. The spleens of all three cases are filled to practically the same degree by large pale-staining cells, which stain positively with the special methods for the more complex lipoids, reactions which indicate that part of the stored material is probably a phosphatid. In the sections of all the spleens only an occasional foam cell is found free in the lumina of the sinuses. The endothelium of the sinuses apparently is uninvolved in the process. The Malpighian bodies are involved quite late in the disease. In the livers of Cases A and B there is practically complete change of the Kupffer cells into the large lipoid containing ones. In Case C, however, in which the child died of pneumococcus meningitis before the process had reached complete development, only some of the Kupffer cells are greatly swollen with lipoid, but practically all of them with Mallory's aniline blue show at least beginning evidences of vacuolization and with Weigert's iron hematoxylin evidences of increased lipoid content. In the lymph nodes the lipoid-containing cells lie outside the sinuses. The clasmatocytes or tissue histiocytes, particularly in the loose fibrous tissue about the various organs, are vacuolated and stain positively for the complex lipoids. The thymus, which was examined in Cases A and C, shows a high lipoid content in the vacuoles of the reticulum cells. The alveoli of the lungs in Cases A and C are filled, sometimes almost completely, with the large lipoid-containing cells. In the lungs of both of these cases the same large cells are present in the loose connective tissue about the vessels, beneath the pleura and in the lumina of the arteries. In the kidney of Case A, large cells are found not only in the capillaries and smaller arteries, but also within the glomerular tufts where some of the cells are of unusual size. There are a few large cells in the bone marrow of Case C and in the smaller vessels in the pancreas.

Our three cases anatomically and microchemically are quite similar to the case described by Niemann and simulate even more closely those described in detail by Knox, Wahl and Schmeisser, and by Siegmund. In spite of the fact that these men believed their cases to be typical examples or precursors of Gaucher's disease, there is prac-

tically no evidence for this, as we have shown above not only from the cases of Gaucher's disease reported here but also from those in the literature. The cells in Gaucher's disease are not so uniformly round, are much more frequently fusiform or elongated and do not give the lipoid-staining reactions of the large cells in Niemann's disease. The substance present in the large cells of Gaucher's disease is apparently insoluble in absolute alcohol, while it is taken out with ease by this solvent in the case of Niemann's disease. Moreover, the cells in Gaucher's disease have a homogeneous, rather refractile cytoplasm which with certain stains presents a peculiar longitudinal striation. In Niemann's type, however, the cells either fresh or when treated with the lipoid stains present a very definitely granular appearance. After treatment with the proper solvents they become highly vacuolated. Perhaps the most striking point of difference lies in the distribution of the cells involved in the two cases. Gaucher's disease with marked regularity involves the pulp and sinuses of the spleen, the periportal connective tissue in the liver and the reticulum cells of the lymph nodes and bone marrow. True Gaucher cells have never been seen outside these situations except in the case of Risel, who found a small collection of such cells in a lymphoid follicle in the thyroid. In Niemann's disease, on the other hand, the large cells with equal regularity are present in the pulp of the spleen, in the thymus, and in the mucosa and submucosa of the intestines; they lie free in the alveoli of the lungs and they are found in the blood vessels of the lungs, kidneys and pancreas; the Kupffer cells of the liver and the tissue histiocytes in general become transformed into the large cells. The types of cells involved are apparently in both conditions members of the reticulo-endothelial system, but the two types of disease show strikingly the differences in reaction of various members of this group.

In experimental hypercholesterolemia Anitschkow [13] was able to produce in the spleen, adrenal, liver, wall of the aorta and in the bone marrow, large cells which contained a vacuolated cytoplasm when stained with the usual technic but which gave markedly positive reactions with Sudan III, Nile blue and the Lorrain Smith-Dietrich method. The fat was not seen in the lymphoid follicles and was present along the adventitia of the arterioles. In one rabbit he noticed that cholesterol and trypan blue were taken up by the same cells. In cases of diabetes with lipemia, in the human, Lutz [14] and

Schultze [11] showed the presence of large lipoid-containing cells in the spleen. Williams and Dresbach [15] in a similar case found the large cells in the liver, lymph nodes and adrenals, and Smith [16] in another case found the lipoid material almost entirely in the spleen but also to a small extent in the liver and lymph nodes. Fahr and Stamm [17] have reported a case in a young girl suffering with a mild atypical diabetes in which the large cells were widely distributed throughout the body. In the lipemia cases the lipoid reactions were very much more difficult to obtain than in the experimental cholesterol-feeding work of Anitschkow. It seems probable that some, at least, of the lipoid material in the lipemia cases is of the nature of the complex lipoids, such as the phosphatids, which are so plentifully present in the cases of Niemann's disease.

A point which we have much difficulty in understanding is the marked involvement of the thymus. Embryogenetically the reticulum cells of the thymus are epithelial in origin. Vital staining experiments have given contradictory evidence as to the ability of these cells to take up vital dyes; the majority opinion is that the thymus does not store them. Aschoff,[18] reviewing the question recently, remarked that the thymus probably can store, but that the point requires reinvestigation. Lubarsch [19] reports the frequent finding of iron and fatty substances in the reticulum cells of the thymus of poorly nourished infants in the first year of life. He therefore suggests that thymic reticulum cells should be included, along with certain other cells, in the reticulo-endothelial system. In any event, it is highly interesting that the thymus had stored large amounts of the lipoid material in five of the six cases examined post mortem. It is perhaps still more striking that the thymus should have taken up this material so much earlier than the usually highly phagocytic Kupffer cells did in our Case C. In this case the lungs were richly supplied with the pale cells and one wonders whether the absence of them from the liver may possibly have been due to their having been swept into the lungs. The finding of the large lipoid-containing cells in branches of the pulmonary artery is more than suggestive of the fact that the foam cells lying free in the alveoli are brought to the lung from the blood stream and removed in the lung. It is possible that some of the cells, too, may have developed from the histiocytes present in the connective tissue of the interlobular septa, about the walls of the bronchi and larger vessels,

because with appropriate stains large numbers of lipoid-containing cells are seen in these situations. Apparently here they are not within lymphatics.

In our Case B of Niemann's disease the condition was diagnosed *in vivo* before operation. A sample of blood taken before operation showed the cholesterol and fatty acid content of the blood to be approximately normal. We have no evidence, from this single determination, therefore, that the disease is due to an upset of some sort in the lipoid metabolism other than the evidence of intracellular lipoid accumulations in widespread locations throughout the body.

The cases described above illustrate most beautifully the selective action of similar cells belonging supposedly to the same general group, for there are certain marked differences between the reactions of these cells in Gaucher's disease, Niemann's disease, diabetic lipemia, experimental cholesterolemia and their response to vital stains. The Gaucher substance is probably cerebrin or an allied substance, while the stored material in Niemann's disease according to Siegmund is probably a phosphatid. Gaucher cells, moreover, take up varying amounts of iron and this material is apparently absent in the cells of Niemann's disease. In any event, the cases of Niemann's disease here reported appear to us to be exquisite examples of a pathologic physiologic storage, in contradistinction to the storage of foreign material such as some of the colloidal dyes. We cannot answer the question as to whether the appearance of the large cells is due to an infiltrative or a degenerative process. We have as yet no evidence of lipemia.

Our Case B improved markedly for a period of two months as a result of splenectomy; it then gradually resumed its preoperative condition.* It is hoped that in the future similar cases will receive more careful study from the point of view of the lipoids of the blood and the effect of feeding various diets. The question as to the value of splenectomy requires further time for final answer.

In addition to the anatomic differences described above, Gaucher's disease clinically stands in sharp contrast to Niemann's disease. Gaucher's disease may start early in life but it extends over a long period of years, while Niemann's disease affects infants, none of

*Since the manuscript was sent to press, information has been received that this child died in the latter part of September, 1925, in another city; so far as we know necropsy was not done.

whom has lived as long as two years. There is a leucopenia in Gaucher's disease and a fairly high leucocytosis in Niemann's disease. Various congenital abnormalities have been associated with the few cases of Niemann's disease on record. There was a mongoloid appearance, with difficulty in respiration in two of the seven cases, and amaurotic family idiocy in a third case. Niemann's disease appears to be a deep-reaching disturbance in metabolism, in which the infants do not thrive and there is a piling up of lipoid material in phagocytic cells throughout the body. Gaucher's disease on the other hand is apparently not accompanied by this profound metabolic disturbance and the individuals so affected may live as long as thirty-nine years, as in Schlagenhaufer's [20] case. Five of the seven cases of Niemann's disease occurred in Jews. Six of these seven were girls and only one was a boy. Both of our cases of Gaucher's disease were Jews and although many of the reported instances of this condition were in Jews, the incidence of this disease is not as highly restricted to this race as it seems to be in Niemann's disease.

As a result of our study we feel that a sharp distinction should be made, in agreement with the work of Pick, between Gaucher's disease and the condition first described by Niemann. Niemann suggested for this disease the name of "large cell metamorphosis of glands." We do not feel that this is a particularly appropriate name in that it connotes nothing of the lipoid material stored in these cells; nor do we think the designation of Pick as "lipoid splenohepatomegaly" especially fitting, because in the first place the process is spread through practically the entire body, and in the second place in one of our cases which was fairly well advanced, the liver was just showing beginning involvement. For the want of a better name we would suggest "lipoid-histiocytosis" as tending to convey the idea of a process involving the storing of lipoid material by the histiocytes throughout the body.

Summary

Two cases are reported, a boy aged 6 years and a woman aged 42 years, in whom there was a history of long-standing increase in the size of the spleen, leucopenia, mild secondary anemia, and epistaxis in one case and uterine bleeding in the other.

Microscopically there are large pale cells which contain iron and give no typical reactions with the usual lipoid stains in the spleen, liver and lymph nodes. These large cells have a marked longitudinal striation with Mallory's aniline blue connective tissue method. These two cases seem to be typical of Gaucher's disease.

Three cases of the condition first described by Niemann are reported in infants aged 18, 14 and 7 months. The history in the first two of these was of long continued disturbance in feeding and a failure of the infants to develop. The third patient died of meningitis at a comparatively early period in the disease. One case was recognized *in vivo* and improved a good deal for two months after splenectomy, but then began to fail again.

Anatomically these cases have a widespread infiltration of lipoid material in the reticulum cells of the spleen, lymph nodes and thymus, Kupffer cells, clasmatocytes of connective tissue, and the large cells in the alveoli and perivascular tissues of the lung. The large foam cells are also present in the arteries of the lungs, pancreas and kidneys, and in the branches of the portal vein. In sharp contrast to the Gaucher cells, these cells stain positively for the complex lipoids, they do not contain iron and are markedly vacuolated after treatment with absolute alcohol. The absence of the large cells from blood smears can probably be explained on the basis of their being filtered out by the capillaries, especially those of the lungs.

Chemical analysis of these spleens is now under way and will be reported elsewhere.

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DESCRIPTION OF PLATES XCVI–C

Fig. 1. Spleen of Case I. Gaucher's Disease. Hemalum and eosin stain. x 240.

Fig. 2. Liver of Case I, showing marked longitudinal fibrillation of the cytoplasm of the Gaucher cells. Mallory's aniline blue connective tissue stain. x 560.

Fig. 3. Thymus of Case A, of Niemann's disease. Foam cells are very close to the Hassall's body. A capillary to the right of the latter is partially filled with a foam cell. Weigert's iron hematoxylin stain. x 560.

Fig. 4. Lung of Case A. This shows several alveoli filled with large pale cells. In the alveolus in the upper left hand corner several of the cells are multinucleated. Hemalum and eosin stain. x 240.

Fig. 5. Liver of Case A. The dark liver cells stand out sharp against the pale cells in the sinusoids. A large foam cell near the center of the field contains two nuclei. Weigert's iron hematoxylin stain. x 560.

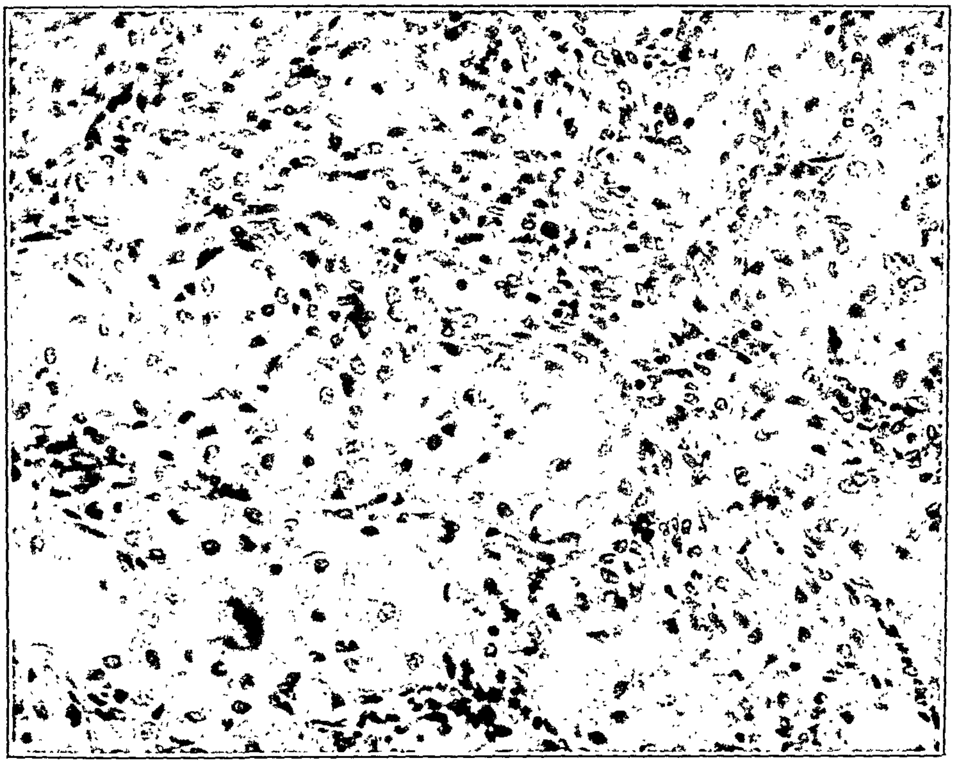
Fig. 6. Spleen of Case A showing the large foam cells in the pulp and the sinuses free of these cells. Delafield's hematoxylin and eosin azur II. x 140.

Fig. 7. Spleen of Case A showing foam cells in pulp and a Malpighian body in the center of the section. The sinusoids stand out clearly and are free of large cells. Mallory's aniline blue stain. x 240.

Fig. 8. Mesenteric lymph node of Case A showing the large vacuolated cells. Heidenhain's iron hematoxylin stain. x 700.

Fig. 9. Kidney of Case A. The right border of the glomerular tuft contains a large foam cell. Another pale cell in the center of the glomerulus is slightly out of focus. Weigert's iron hematoxylin stain. x 560.

Fig. 10. Spleen of Case B of Niemann's disease. The large cells stain an intense black. A few of them are seen about the arteriole in the center of the field. The sinuses are free of large cells. Frozen section after formalin fixation. Weigert's iron hematoxylin stain. x 60.



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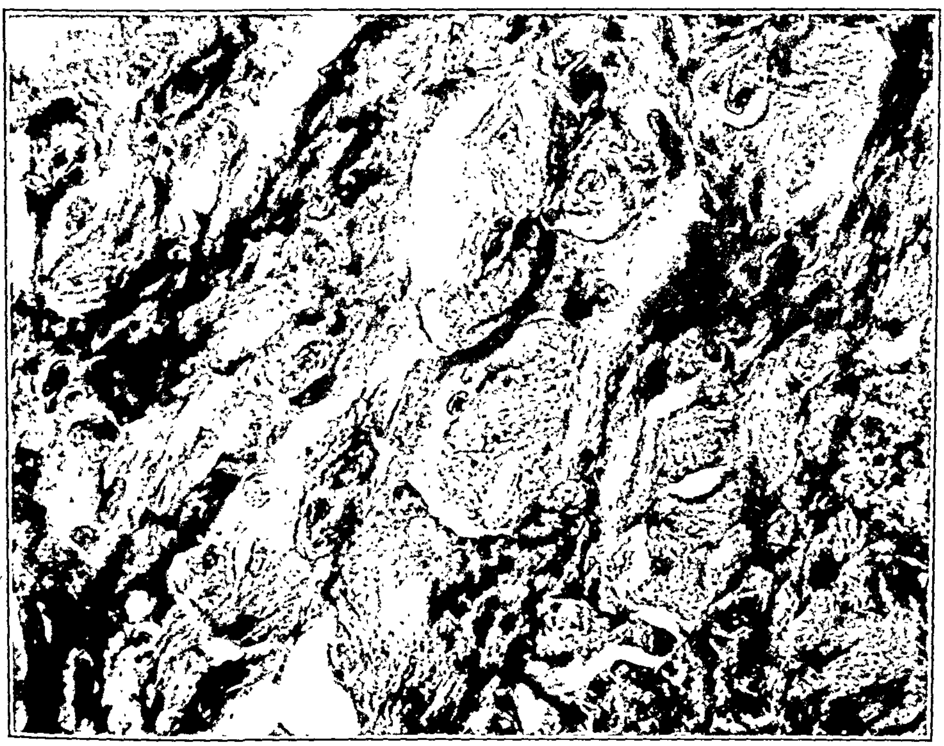

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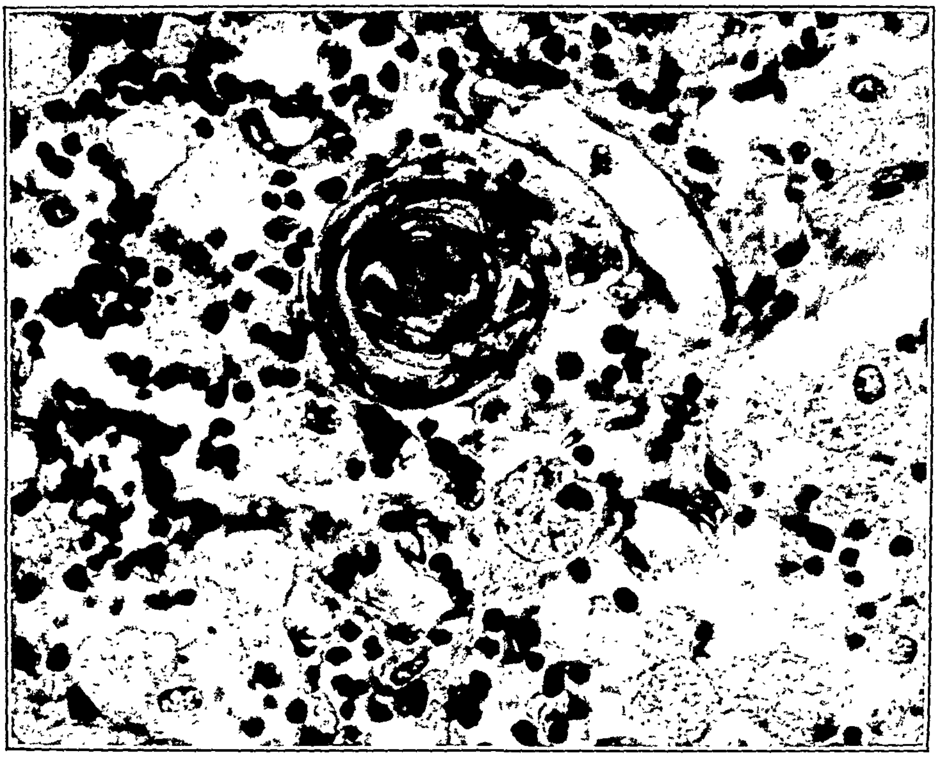

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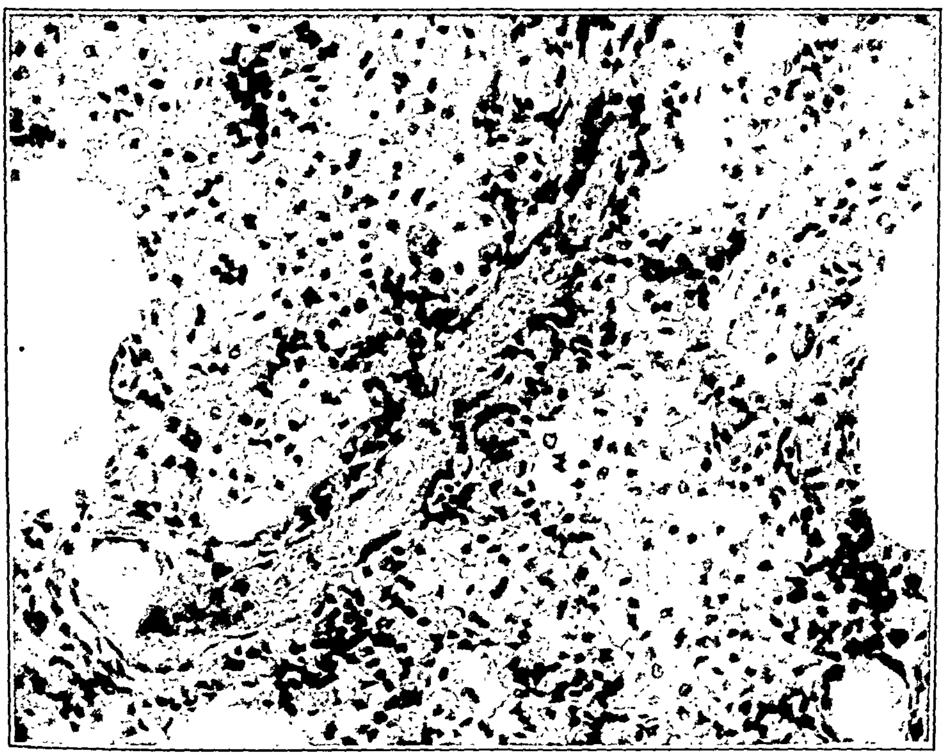

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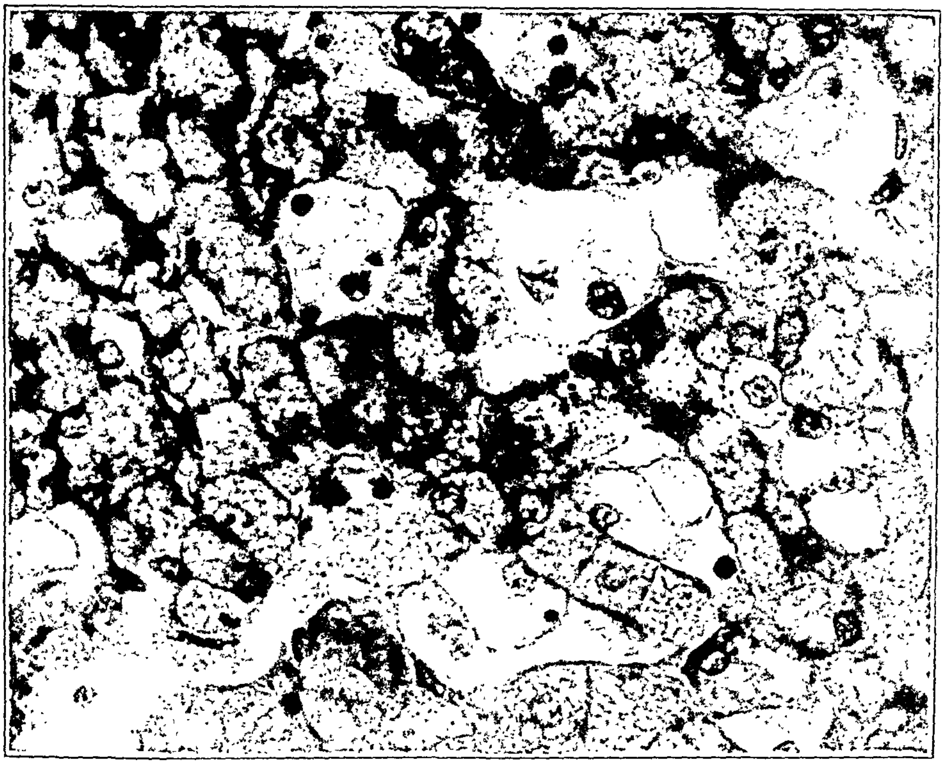

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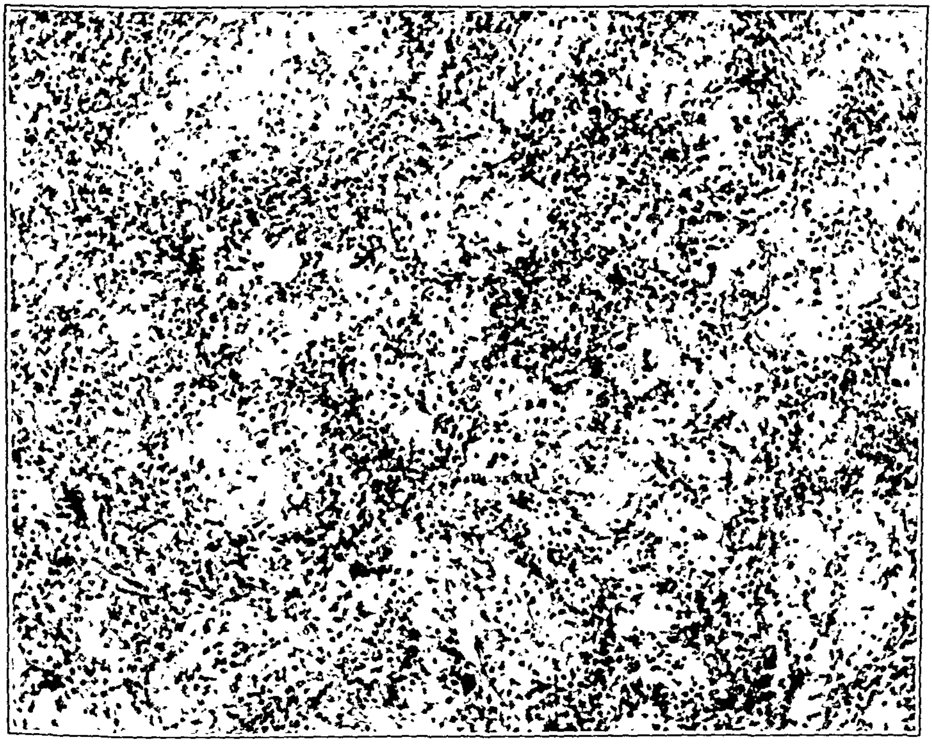

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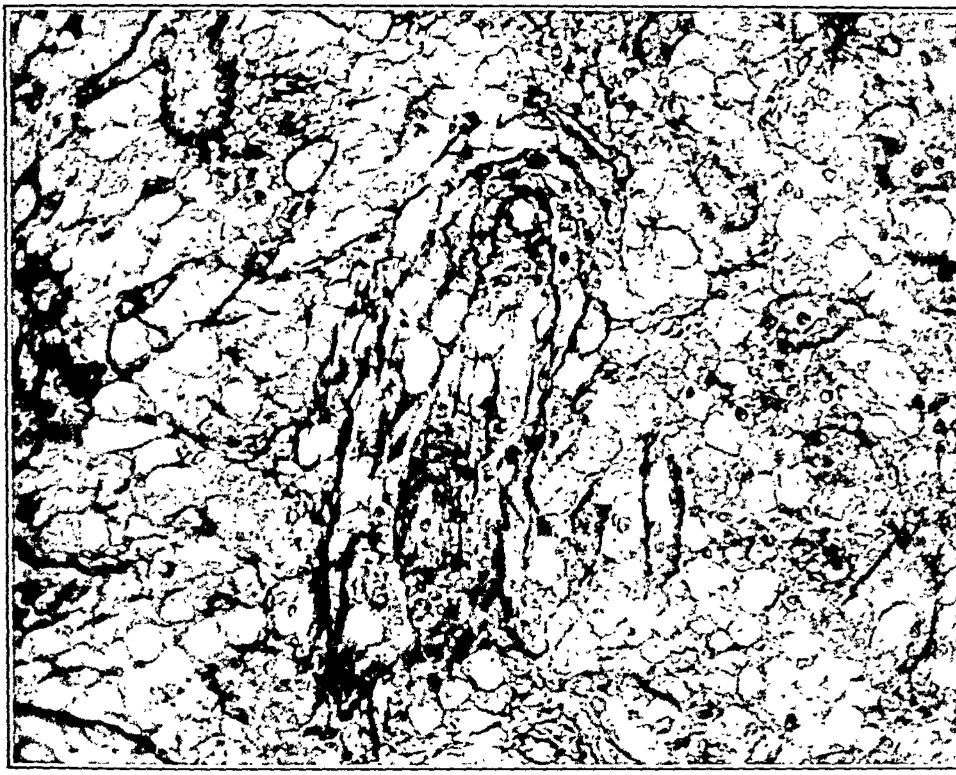

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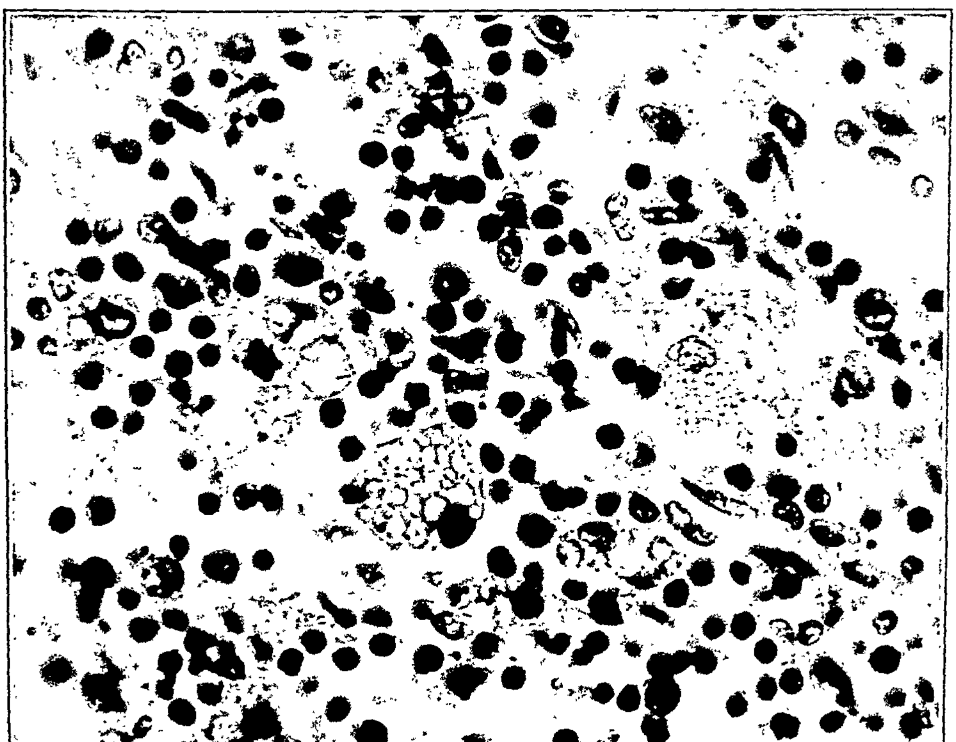

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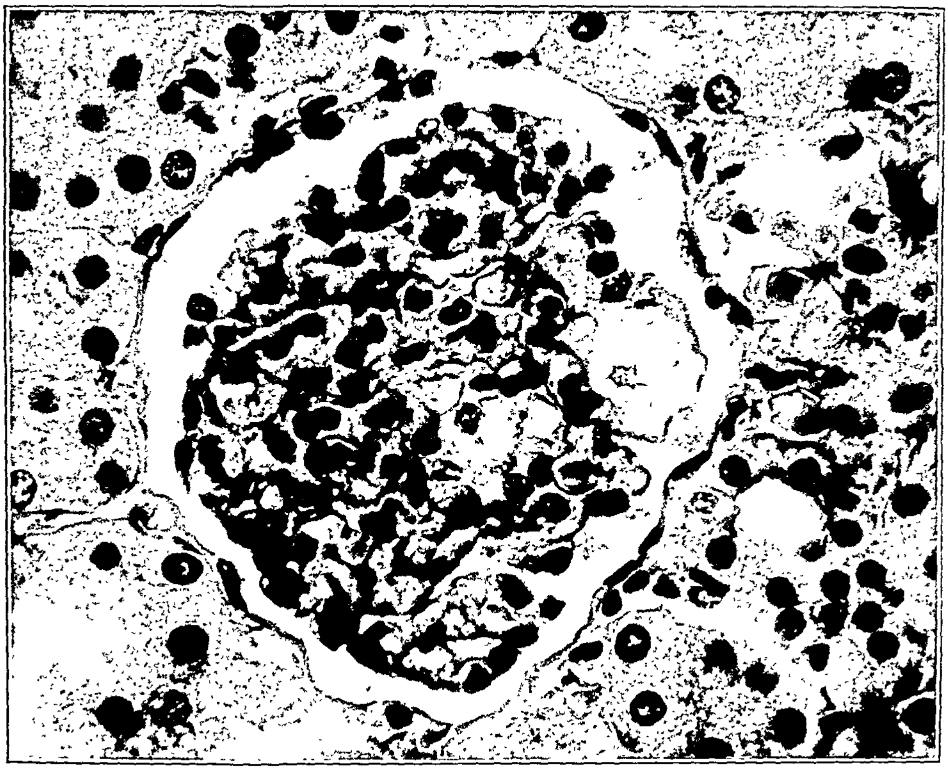

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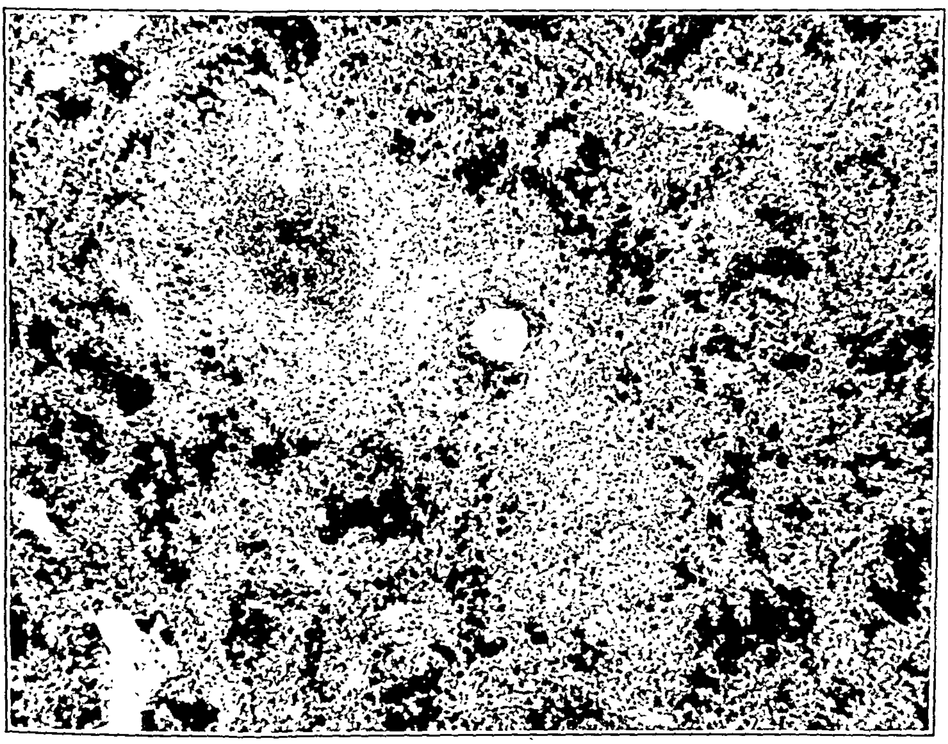

10

EPENDYMOMAS OF THE LATERAL AND FOURTH VENTRICLES OF THE BRAIN *

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Differences are now recognized between tumors arising from the ependyma and those originating from the choroid plexus. This distinction, however, has not been made in many of the reports given in the older literature, although some are excellent and represent good work. Of these older reports some do not mention the important cell characteristics which differentiate between tumors of choroid plexus and those of ependymal origin, while others deny any possibility of distinguishing between cells arising from these different places. This confusion makes almost impossible an accurate summary of the ependymomas now on record. Then, too, for example, the tumor of the brain and cord originally described by Spiller [1] as a glio-sarcoma was later regarded as an ependymoma of the fourth ventricle. There are reports which suggest that the tumor probably is an ependymoma described under another name.

In 1914 Natonek [2] arranged in two groups the tumors of ependymal and choroid plexus origin recorded in the literature:

(1) Tumors of the choroid plexus epithelium
  (a) normal structure
  (b) modified structure

(2) Tumors of the ventricle ependyma
  (a) normal position
  (b) misplaced

This classification in the first group is based on histologic structure but in the second on topography only. The choroid plexus tumors of normal structure are the benign papillomas and those of modified structure are carcinomas of which Natonek thinks there are three, namely: (1) Kaufmann's [3] adenocarcinoma of the lateral ventricle which is described as resembling a malignant bowel polyp, (2) Roki-

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tansky's[4] colloid carcinoma of the choroid plexus and (3) Wunscheim's[5] tumor of the fourth ventricle. As characteristics of the ependymal epithelial tumors Natonek emphasizes their multiplicity (local metastases) and their ability to infiltrate. He believes that the one reported by Saxer[6] is the only non-malignant tumor of this kind. All the other ependymal tumors according to him are termed carcinomas and he refers specifically to those reported by Spät,[7] Kolpin,[8] Hart,[9] Cornil,[10] and Bielschowsky and Unger.[11]

Not all the tumors as originally discussed by the authors mentioned come into such a grouping. Kaufmann, for example, states that the tumor he describes had its origin in the ependyma of the lateral ventricle.

Ribbert's[12] paper in 1910 on neuroepithelium in gliomas of the brain and spinal cord mentions similar tumors reported by Stroebe, Saxer, Stolpe, Chiari, Henneberg, and Muthmann and Sauerbeck,[13] and he describes a tumor of the fourth verticle which he says is like the description given by Muthmann and Sauerbeck. This one reported by them, another by Spiller[1] and three by Mallory[14] are considered by Bailey[15] as the only five authentic ependymomas recorded in the literature. He adds six more.

According to Bailey's statements, ependymal cells of the embryo are ciliated, and at the base of each cilium is a small granule known as blepharoplasten. This may be a single granule, a pair of granules one above the other, a minute rod or a dumb-bell-shaped form. For a short period during embryonic life blepharoplasten granules exist in the choroid cells of the lateral ventricles. They are transient in most ependymal cells, but persist in the adult in the cells of the aqueduct of Sylvius, of the rhomboid fossa and of the central canal of the spinal cord. Blepharoplasten granules, according to Bailey, were observed in the ependymal cells by Marchi and were described by Von Engelmann. Weigert subsequently described them in his monograph on the neuroglia to which Mallory refers when he suggests that the presence of these granules is important in establishing the ependymal origin of certain tumors of the brain. Such granules may be demonstrated by any neuroglia stain but Mallory recommends phosphotungstic acid hematoxylin. Bailey states that neuroglia fibrils are formed normally from the ependyma even in postfetal life, and that possibly this mode of growth has a large rôle in certain pathologic conditions.

From these statements, the finding of blepharoplasten granules in cells and of glia fibrils is important in recognizing tumors of ependymal origin. Such a distinguishing feature has not been considered in the description of many of these brain tumors.

Bailey's conception of ependymomas is that they grow slowly and are relatively benign. They should not be removed, he believes, when demonstrated at operation. He reports a patient in excellent health more than ten years after one of these tumors had been demonstrated by operation but left undisturbed. This benign course, however, is not true in every instance, and probably there need be considered two varieties, the one benign, the other malignant with infiltrative qualities, metastases and rapid growth. Just where to make the distinction may sometimes be difficult.

Mallory has described these tumors as gliomas of ependymal origin, while Ribbert speaks of them as gliomas containing neuroepithelium. These designations specify the presence of glia tissue along with the epithelium. Spiller and Bailey use ependymoma as a descriptive name which seems sufficiently simple and accurate when amplified by terms designating benign or malignant qualities.

Histologically the ependymomas are essentially papillary tumors. The individual papilla consists of a central stalk, chiefly a thin-walled blood vessel and very little fibrous tissue covered by several layers of polygonal cells with granular cytoplasm. Where the tumor growth assumes malignant qualities there is a dense palisade of cells several layers deep arranged radially and at right angles to the vessel lumen. In addition, duct-like structures are present in variable numbers. Between the cell masses are delicate or coarse glia fibrils, the number of which varies considerably. Blepharoplasten granules are present in the cytoplasm of the cells. Many of the tumors reported are primary in the fourth ventricle of the brain.

These comments are made as introductory to a report of two ependymomas, one of the left lateral ventricle, the other of the fourth ventricle. The latter ependymoma resembles both grossly and microscopically the tumors of the fourth ventricle reported by Bailey.

G. A., a male 16 years of age, was in good health according to statements by relatives until September, 1923, when he injured the lumbar and sacral region of his back in falling from a tree. An examination by a physician at this time demonstrated no significant injury, but the boy did not return to school,

although he was up and about most of the time. In March, 1924, stiffness of the legs was noted, chiefly difficulty in obtaining full extension. After a month without improvement, tuberculosis of the spine was diagnosed. For a year an afternoon temperature of about 99.6 F. was observed. About ten days before admission to the hospital his physical condition grew worse with loss of appetite and morning attacks of nausea and vomiting. On January 10th, these attacks became so severe that no liquids or solids could be kept in the stomach. His mental state changed in that he was surly, his memory failed and he was disoriented. Spasticity of the legs increased. For nine months there had been blurred vision. The boy was admitted to St. Luke's Hospital, Chicago, Jan. 11, 1925, to the orthopedic service of Dr. E. W. Ryerson and later to the medical service of Dr. A. R. Elliott. The nausea and vomiting continued, emaciation became marked, mental deterioration increased and death occurred March 8th, without a satisfactory clinical diagnosis having been made.

The essential changes demonstrated by necropsy of the trunk, head and neck are confined to the brain in which there is a huge tumor of the left cerebral hemisphere. The description of the tumor follows.

The formalin hardened brain on March 19, 1925, weighs 1616 gm. The right cerebral hemisphere is 17.5 cm. long and 7 cm. wide and the left is 18.5 cm. long and 8 cm. wide. The cerebral convolutions are markedly flattened, especially of the left in its posterior two-thirds. On the medial surface of this left lobe beginning 3 cm. in front of the posterior pole, extending forward 4.5 cm. and vertically 5.5 cm. to the superior surface is a friable gray and white mass which here has completely replaced the brain tissue. Close to the midline on the superior surface and continuous with it is an irregular nodule bulging from the cortex and about 2 cm. in diameter. Slightly in front of this and 5 cm. from the midline is another mass 3.5 x 4 cm. The dura is densely adherent to these places and the vessels covering them are markedly dilated with blood. The leptomeninges otherwise are moderately hyperemic, lie closely in the sulci of the brain and are semitransparent. Immediately behind the optic chiasma and encroaching upon it is a gelatinous gray mass, cone-shaped with its base corresponding to the tip of the infundibulum. This tissue is 2 cm. in diameter at its base and is about 2 cm. high. The aqueduct of Sylvius is patent and pushed slightly to the right of the midline. There are no changes in the lining or plexus of the fourth ventricle. The third ventricle is unchanged except that it has been pushed to the right side of the midline by the tumor mass in the left half of the cerebrum. The right lateral ven-

tricle is markedly distended, the maximum lateral dimension is about 3.2 cm. and the vertical 5.4 cm. Of the left lateral ventricle there is present only the anterior 5 cm. of the cornu anterior and the anterior 1.5 cm. of the cornu posterior. From a coronal section at a level corresponding to the anterior end of the third ventricle and extending backward practically to the posterior tip of the left cerebral hemisphere, a length of about 12 cm. and about 7 cm. in both of its other dimensions, there is a huge tumor (see Figs. 1 and 2) which has entirely replaced the brain substance. In the same regions it has destroyed the cortex. The tumor is soft, easily torn, and yellow and white mottled with old and recent hemorrhages. It contains irregular cyst-like regions, the largest about 2 cm. in diameter, filled with a gelatinous white substance. Portions of the yellow tissue are necrotic. The tumor substance contains many blood vessels of which the largest has a lumen about 4 mm. in diameter and a wall thick with hyaline fibrous tissue. The tumor tissue has replaced the lateral ventricle except the portions mentioned. The choroid plexus of the right lateral ventricle is unchanged, but of the left only the anterior portion remains and it lies on the surface of granular tumor tissue which extends almost to the opening into the third ventricle. The lining of the left lateral ventricle in front is studded with many small masses of similar tissue. One of these on the septum pellucidum is about 15 mm. in its surface dimensions and protrudes 5 mm. In the right lateral ventricle are scattered others from 5 to 8 mm. in diameter. A probe passed through the opening of the lateral ventricle into the third ventricle continues into the tissue mass in the region of the infundibulum. In the coronal section through the right cerebral hemisphere in front of the corpora quadrigeminae there is a gray tumor mass exposed in the temporal lobe immediately beneath the floor of the genu posterior of the ventricle (see Fig. 1). It is about 1.5 cm. in diameter and extends through the brain tissue to the pia.

Thin sections of the tumor tissue prepared from paraffin blocks, stained with hematoxylin and eosin and with phosphotungstic acid hematoxylin, contain tumor tissue with a papillary structure (see Fig. 3). In the center of each papilla is a thin-walled blood vessel on which are four to six layers of polygonal cells, surrounded by a palisade about equally wide of cells more densely arranged and at right angles to the central vessel. Glia fibrils and blepharoplasten

granules (see Fig. 4) are present in the cells stained with phosphotungstic acid hematoxylin. By careful search a few tubules (see Fig. 5) lined with a single layer of cuboidal or low columnar epithelium were found. The papillary structure is present also in the smaller tumor masses of the ventricles, right cerebral hemisphere and infundibulum.

For the second ependymal tumor reported we are indebted to the courtesy of Dr. Dean Lewis. It occurred in a white man 34 years of age, who complained of headache, nausea, vomiting, disturbance of vision and irritability and symptoms, beginning about fifteen months before death, attributed to a brain tumor, possibly of the hypophysis. Three weeks before death a right temporal decompression was done by Dr. Lewis. At the operation a markedly increased intracranial pressure was observed and following the operation brain tissue herniated through the decompression opening of the skull. Death occurred suddenly with severe difficulty in respiration. A postmortem examination of the head only was permitted. Excepting trauma of the cranium, pericranial tissues and brain resulting from the operation, the changes of particular note are confined to the fourth ventricle and the adjacent tissues.

The brain without the dura on July 20, 1925, weighs 1514 gm. The right cerebral hemisphere has the following dimensions: length 17.5 cm., width 7 cm. and thickness 7.5 cm. The respective dimensions of the left are: 18 cm., 8 cm. and 8 cm. The leptomeninges are gray and slightly opaque, the convolutions flattened and the sulci correspondingly narrow. On the right cerebral hemisphere corresponding to the upper end of the fissure of Rolando there is an irregular torn mass of brain substance 4 x 5 cm. in which there are recent hemorrhages occupying about one-fourth of the tissue. This mass projects irregularly from the brain cortex about 2.5 cm. In segments of the brain made in the usual way by cutting in parallel coronal planes about 1.5 cm. apart, there is a slight widening of the left lateral ventricle and the septum pellicidum is pushed to the right. The third ventricle is widely distended by a blood clot which is continuous behind and through the aqueduct of Sylvius into the fourth ventricle. Wedged between the dorsum of the spinal cord and the inferior surface of both lobes of the cerebellum and pushing these structures apart is a smooth gray-white tumor mass 4.5 cm. long, about 3.5 cm. wide and 3.5 cm. thick (see Fig. 6) arising from the

velum posterior. The anterior end of this mass is continuous with the blood clot filling the fourth ventricle and about 2.5 to 3 cm. in its greatest dimensions. On section the tumor tissue is gray-white, friable and mottled with many pin-point and slightly larger hemorrhages. On its dorsal surface immediately below the lobes of the cerebellum the thin fibrous capsule covering this mass is densely adherent to the dura and in removing the brain from the cranium the tissue is torn over an area 2 x 1.5 cm. Adjoining this place there is an erosion of the bone forming the posterior part of the foramen magnum.

Sections stained with hematoxylin and eosin contain cells polygonal in outline in the less dense regions but compressed to a spindle shape in the compact places. There are many thin-walled capillaries (see Fig. 7). The cytoplasm is faintly granular and abundant, the nuclei are vesicular and usually have a single round mass of chromatin close within the nuclear membrane. In sections stained with phosphotungstic acid hematoxylin there are blepharoplasten granules in the cell cytoplasm, but only a few glia fibrils. Most of the cells are relatively immature but in some the cytoplasm is continued into thread-like prolongations in one or more places.

Summary and Conclusions

Two ependymomas of the brain are reported. One, primary in the lateral ventricle, has the infiltrative qualities of a malignant tumor, while the other, in the fourth ventricle, is chiefly an expansile growth pushing aside the adjacent tissues. This difference in growth suggests that certain ependymomas are benign while others are malignant, and that growth characteristics should be recognized in the general class of tumors arising from the ependyma.

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DESCRIPTION OF PLATES CI–CIII

FIG. 1. Photograph illustrating the tumor size in the left cerebral hemisphere at the level of the corpora quadrigeminae, and the tumor metastasis in the right temporal lobe.

FIG. 2. Photograph illustrating the tumor size and appearance in a coronal plane through the middle of the left occipital lobe.

FIG. 3. Photomicrograph to illustrate the palisade arrangement and papillary structure. X 100.

FIG. 4. Photomicrograph illustrating glia fibrils and blepharoplasten granules. X 1200.

FIG. 5. Photomicrograph of the ducts contained in the ependymoma of the lateral ventricle. X 200.

FIG. 6. Photograph of the ependymoma of the fourth ventricle.

FIG. 7. Photomicrograph illustrating the tissue structure of the fourth ventricle ependymoma. X 140.

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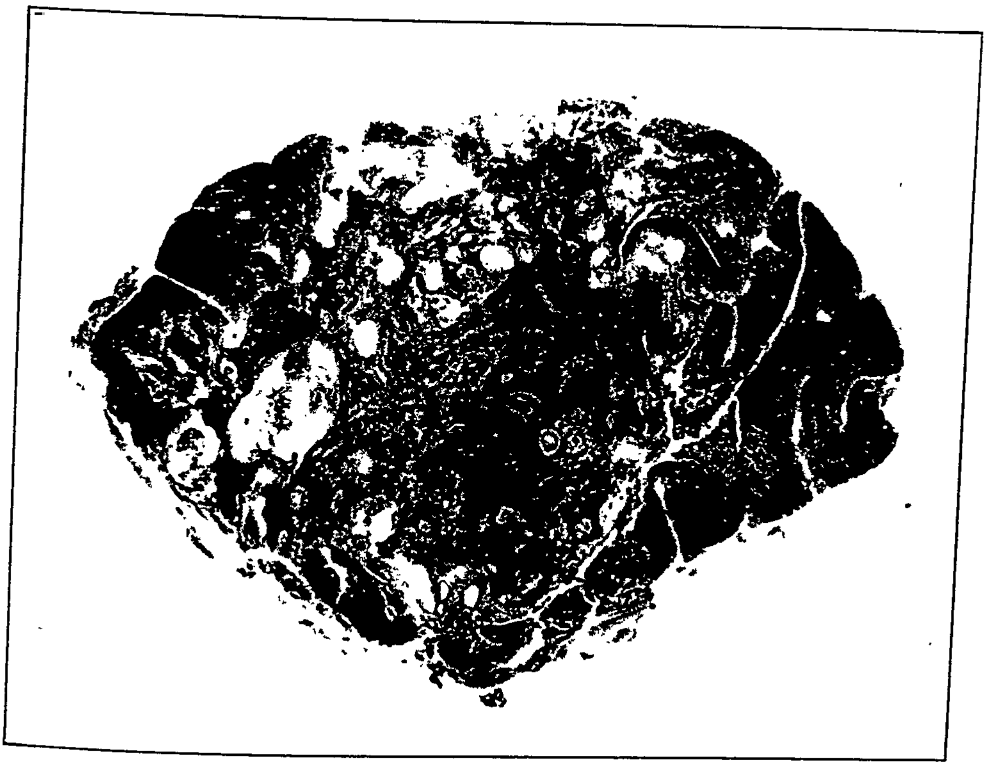

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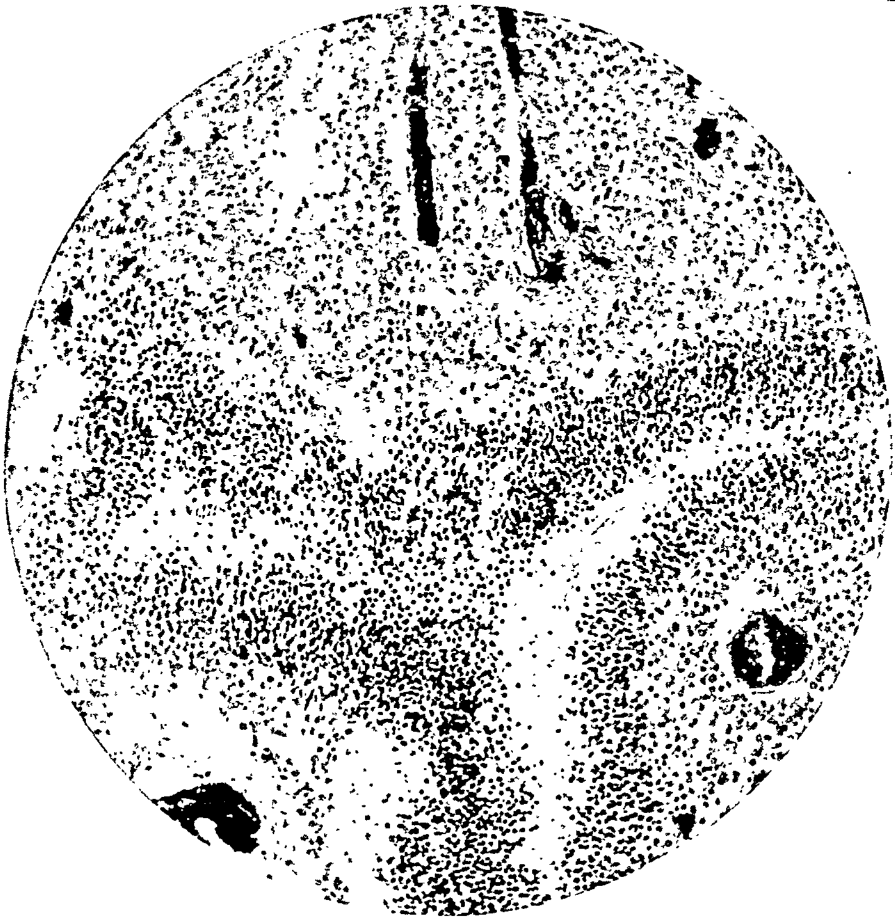

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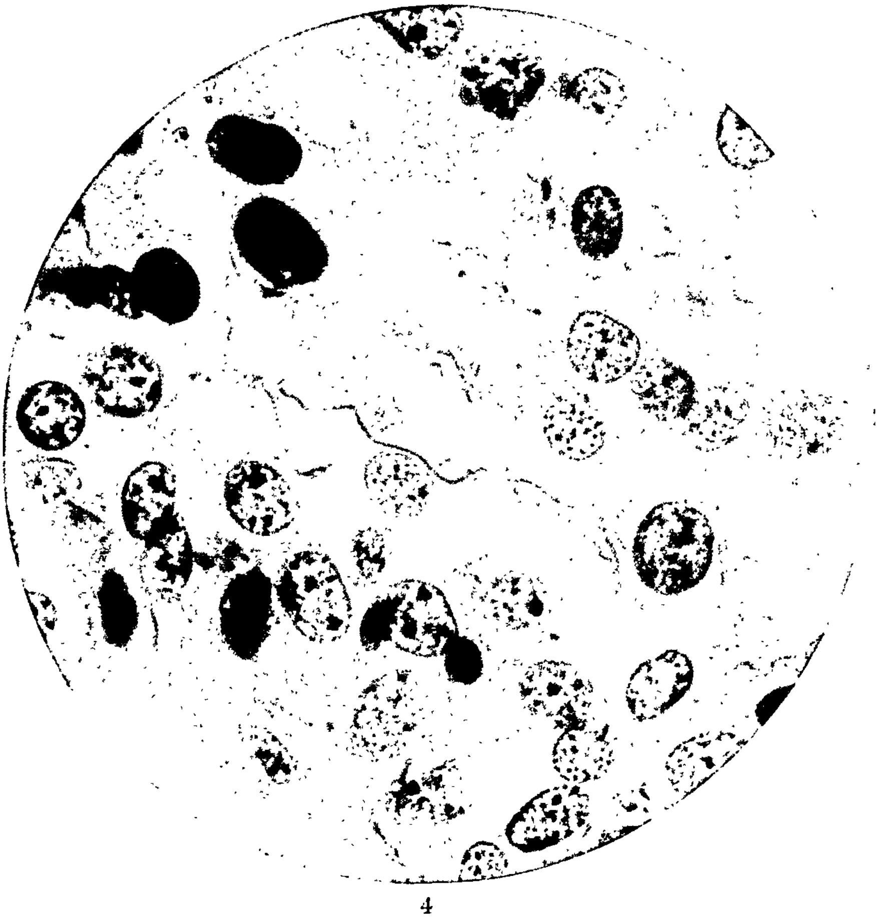
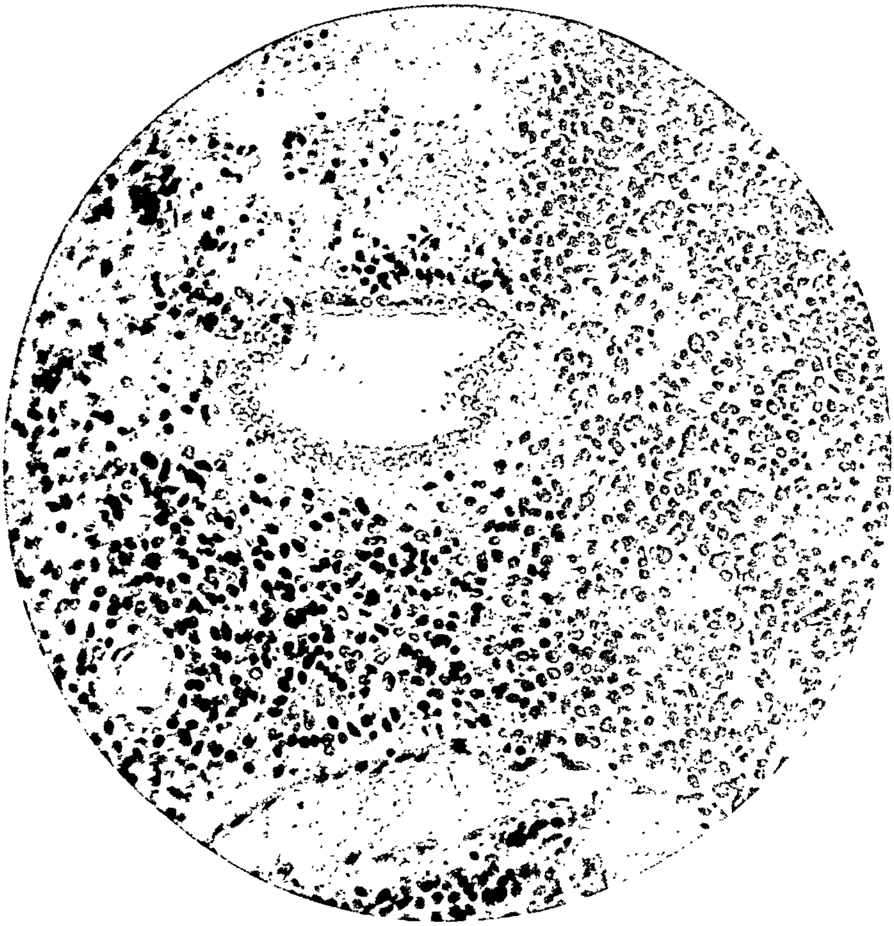


4

Hirsch and Elliott

Ependymomas



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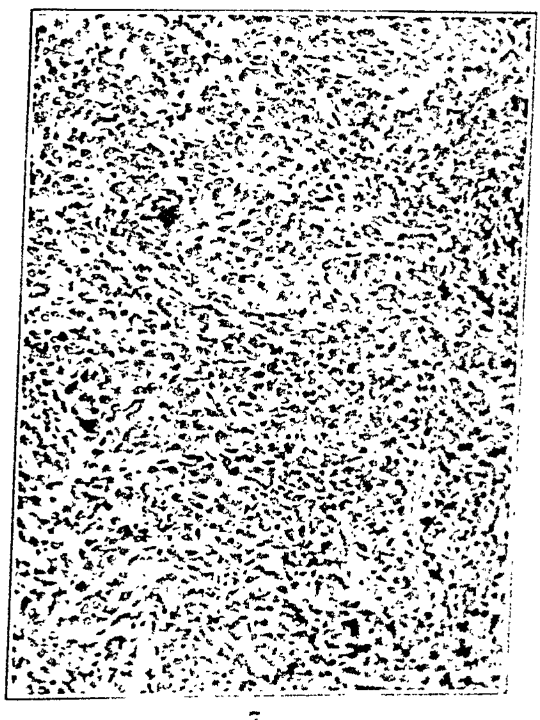

7

RESEARCHES ON VACCINE VIRUS *IN VITRO* WITH SPECIAL REFERENCES TO ITS AFFINITY FOR NERVE TISSUE *

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It is well known that when a piece of fresh tissue is put at the bottom of a culture tube, chemical reduction of the culture medium takes place and at the same time several substances dialyze out of the tissue. However, the effect of tissue on micro-organisms under cultivation is still not understood.

The present study was undertaken with two objects in view, first to prove the effect of the tissue on vaccine virus in culture medium; and second to determine whether or not vaccine virus migrates along the tissue *in vitro*.

EXPERIMENTAL METHODS AND DATA

I. COMPARISON OF THE AFFINITY OF VACCINE VIRUS FOR THE KIDNEY AND THE SPINAL CORD

The cornea, skin and testicle of rabbits are suitable places for the growth of vaccine virus *in vivo*, of which the testicle seems to be the most favorable. This has been already proved by the experiments of Noguchi [1] and Henseval.[2] Recently Marie [3] reported that when the virus is inoculated into the brain of a rabbit, it causes the death of the animal after four or five days. Also Levaditi and Nicolau [4] showed that if vaccine virus which had been passed several times through rabbits by intratesticular inoculation is injected into the brain of a rabbit, it causes a fatal infection and they have succeeded in making passages from brain to brain in series. I have found that when a strain of vaccine virus which has been passed through rabbits by intratesticular inoculation without increasing its virulence, is injected into the brain of a rabbit, its virulence increases very quickly. In view of the above facts, there seems to be no doubt that the central nervous system is an especially suitable place for the propagation of vaccine virus. On the contrary, according to Ohta-

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wara [5] and Levaditi and his co-workers, the affinity of the virus for the kidney is said to be either completely lacking or else very slight *in vivo*.

Therefore, I have carried out the following experiments in order to obtain some knowledge as to the affinity of vaccine virus *in vitro* for these tissues, namely, kidney, spinal cord and testicle.

The culture medium employed throughout the experiments on the affinity of virus for tissue was 3 per cent glucose broth containing 1 per cent glycerin with a pH of 7.0 to 7.2.

In every instance the material employed for inoculation into culture medium was the testicular strains of vaccine virus which had been passed several times through rabbits. The stock emulsion of the virus was prepared by grinding up aseptically removed vaccinated testicle with 50 per cent glycerin solution containing 0.5 per cent carbolic acid in the proportion of 1.0 gm. of the tissue to 2.5 or 5.0 c.c. of the solution. After grinding, this emulsion was aspirated with a sterile pipette through sterile gauze and put into a sterile test tube. This test tube was allowed to stand in an icebox for a few days, thus permitting the coarse particles to settle. For the purpose of inoculation the supernatant glycerin was pipetted off and varying dilutions, 1: 2 or 1: 5 in physiologic salt solution, were prepared.

The method employed consisted in inoculating medium from two series of test tubes containing 10 c.c. of the culture medium mentioned above and a piece of fresh sterile tissue (either spinal cord or kidney) at the bottom. Into each tube 0.1 c.c. of the diluted vaccine virus was placed. After inoculation, the tubes were covered with a layer of sterile paraffin oil and placed in an incubator at 37 C. Every twenty-four hours during incubation the test tubes of this series were taken out of the incubator one at a time, and if the culture medium was free of contaminating bacteria, it was transferred into another sterile tube with a capillary pipette. The tissue at the bottom of each tube was washed several times with sterile salt solution and weighed to the milligram. An emulsion of the washed tissue was made by grinding with sterile salt solution in the proportion of 0.1 gm. of the tissue to 1.0 c.c. of salt solution, *i.e.*, 1: 10 dilution of the tissue. For the purpose of inoculation into a rabbit, dilutions of the culture medium and the emulsion of tissue were prepared with sterile salt solution as follows: culture medium, 1: 1, 1: 10 and 1:100; tissue, 1: 10 (original emulsion), 1: 100 and 1: 1000.

The presence of the virus in the various dilutions of culture medium and tissue was tested according to Groth's [6] method of intradermal inoculation, *i.e.*, 0.1 c.c. of each inoculum was injected intradermally into a normal rabbit previously shaved over the sides of the abdomen. Altogether twelve inoculations were made at one time at least an inch apart in a single animal. The rabbits were white healthy adults weighing usually from 1.5 to 2.0 kg. The emulsion of spinal cord and the spinal cord medium were injected on the left side and the emulsion of kidney and the kidney medium on the right side. Although an effort was always made to prevent any of the inoculum escaping into the subcutaneous tissues, it is likely that in some instances this happened to some extent. After inoculation, the skin was observed daily for signs of reaction until inflammatory processes had nearly disappeared.

The results of the experiments are shown in Table I. This table shows clearly that vaccine virus has an affinity for the spinal cord of rabbits but almost no affinity for the kidney. Twenty-four hours after inoculation, the virus was found to be present in both the

TABLE I

Affinity of Nervous Tissue for Vaccine Virus

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				2	3	4	5	6	7
117	24 hours	Emulsion of spinal cord	1:10	+	+	++	++	++	R
			1:100	+	+	++	++	++	R
			1:1000	−	+	++	++	++	R
		Fluid medium	1:1	+	+	+++	+++	+++	R
			1:10	+	+	+++	+++	+++	R
			1:100	?	+	++	++	++	R
		Emulsion of kidney	1:10	?	+	++	++	++	R
			1:100	−	+	++	++	++	R
			1:1000	−	−	+	+	++	R
		Fluid medium	1:1	?	+	+++	+++	+++	R
			1:10	?	+	+++	+++	+++	R
			1:100	−	+	++	++	++	R

+ = visible lesion. The number of plus marks represents the size of the lesion, +++ being the maximum.
− = absence of a visible lesion.
R = inflammatory process retrogressing.

TABLE I (*continued*)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin: 2	3	4	5	6	7
118	2 days	Emulsion of spinal cord	1:10	+	++	++	+++	+++	R
			1:100	+	++	++	++	++	R
			1:1000	−	+	+	+	+	R
		Fluid medium	1:1	+	+++	+++	+++	+++	R
			1:10	+	+++	+++	+++	+++	R
			1:100	+	+	+	++	+++	R
		Emulsion of kidney	1:10	−	−	−	+	+	R
			1:100	−	−	−	+	+	R
			1:1000	−	−	−	−	−	R
		Fluid medium	1:1	+	+++	+++	+++	+++	R
			1:10	+	+++	+++	+++	+++	R
			1:100	+	+	+	+	+	R
119	3 days	Emulsion of spinal cord	1:10	+	++	++	++	R	
			1:100	+	+	+	+	R	
			1:1000	+	+	+	+	R	
		Fluid medium	1:1	+	+++	+++	+++	R	
			1:10	+	++	++	++	R	
			1:100	+	+	+	+	R	
		Emulsion of kidney	1:10	−	−	−	−	−	
			1:100	−	−	−	−	−	
			1:1000	−	−	−	−	−	
		Fluid medium	1:1	+	++	++	+++	R	
			1:10	+	+	+	+	R	
			1:100	−	+	+	+	R	
120	4 days	Emulsion of spinal cord	1:10	+	+	++	++	++	++
			1:100	+	+	+	++	++	++
			1:1000	−	?	+	+	+	+
		Fluid medium	1:1	+	+	++	+++	+++	+++
			1:10	−	?	+	++	++	++
			1:100	−	?	+	++	++	++
		Emulsion of kidney	1:10	−	−	−	?	?	+
			1:100	−	−	−	−	−	−
			1:1000	−	−	−	−	−	−
		Fluid medium	1:1	+	+	++	+++	+++	+++
			1:10	+	+	+	++	++	++
			1:100	−	?	+	++	++	++

spinal cord and the kidney tissue, each giving similar skin reactions; at the end of forty-eight hours' incubation in the test tube the virus in the spinal cord caused marked inflammatory processes in the skin, and four days later was found to be still active, whereas the incubated kidney showed a marked decrease in virus and an earlier disappearance.

The papules produced by the virus incubated in spinal cord were different from those caused by the virus in kidney or by the fluid portion of either medium. The papules during the active stages of the infection were redder and more prominent, the infiltration more intense and profound. Later they were sometimes covered with a crust which often remained for weeks.

The culture medium containing the spinal cord produced a more active reaction on the skin than that containing the kidney although the difference was by no means marked.

Repetitions of this experiment have in all cases given similar results.

The intradermal injection of an emulsion of a normal spinal cord caused no inflammatory reaction in the skin of rabbits.

Effect of Nervous Tissue on Vaccine Virus of Low Virulence

Vaccine virus of the lot previously used was employed in this experiment. It showed, however, a definite decrease of virulence in consequence of long preservation. Thus the inoculation of 0.1 c.c. of 1:100 dilution into the skin of a rabbit produced a definite though not intense inflammatory reaction.

The methods and culture medium employed to determine the effect of nervous tissue on virus of low virulence are similar to those previously used. The results of the experiment are summarized in Table II. It is quite evident that the skin reacted to the 1:10 tissue emulsion, while the medium failed to produce a visible lesion, although the medium of two days' incubation produced a papule. In this experiment, the vaccine virus incubated with the spinal cord for twenty-four hours produced a papule earlier than when incubated a longer time; its inflammatory processes, however, were not intense. On the contrary, the spinal cord which was incubated with the virus two or three days caused a papule later, but its infiltration was more intense and extensive.

TABLE II

Effect of Nervous Tissue on Vaccine Virus of Low Virulence

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin					
				5	6	7	8	9	10
104	24 hours	Emulsion of spinal cord	1:10	?	+	+	+	R	
			1:100	−	−	−	−	−	−
			1:1000	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	−	−
			1:10	−	−	−	−	−	−
			1:100	−	−	−	−	−	−
105	2 days	Emulsion of spinal cord	1:10	−	−	?	+	+++	+++
			1:100	−	−	−	−	−	−
			1:1000	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	++	+++
			1:10	−	−	−	−	−	−
			1:100	−	−	−	−	−	−
106	3 days	Emulsion of spinal cord	1:10	−	−	−	−	+	+++
			1:100	−	−	−	−	−	−
			1:1000	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	−	−
			1:10	−	−	−	−	−	−
			1:100	−	−	−	−	−	−

For legend see Table I.

The results indicate that the virus survives longer in the nervous tissue than in the medium, and it would appear that the virus diminishes in amount after incubation but increases in virulence.

II. Comparison of the Affinity of Vaccine Virus for the Testicle and the Spinal Cord

The incubation was carried out at 37 C. and at room temperature (about 21 C.). In the case of the incubation at room temperature I gave my attention to obtaining evidence as to whether the tissue, especially the spinal cord at the bottom of the culture tube, had any effect on the virus suspended in the medium, simultaneously with my efforts to compare the affinity of the virus for the testicle and spinal cord.

TABLE III

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at 37 C.)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin 2	3	4	5	6	7
30	24 hours	Emulsion of spinal cord	1:10	+	+	++	++	R	
			1:100	−	+	+	++	R	
			1:500	−	−	−	+	R	
		Fluid medium	1:1	+	++	+++	+++	R	
			1:10	?	+	+++	+++	R	
			1:50	−	−	++	++	R	
		Emulsion of testicle	1:10	−	+	++	++	R	
			1:100	−	−	?	+	R	
			1:500	−	−	−	+	R	
		Fluid medium	1:1	+	++	+++	+++	R	
			1:10	+	+	+++	+++	R	
			1:50	−	−	++	++	R	
31	2 days	Emulsion of spinal cord	1:10	−	−	+	+	++	+++
			1:100	−	−	−	+	+	++
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	+	++	+++
			1:10	−	−	−	+	++	+++
			1:50	−	−	−	−	+	++
		Emulsion of testicle	1:10	−	−	−	−	?	++
			1:100	−	−	−	−	−	−
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	+	+	++	+++
			1:10	−	−	−	+	++	+++
			1:50	−	−	−	−	+	+
32	3 days	Emulsion of spinal cord	1:10	+	++	++	R		
			1:100	+	+	+	R		
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	−	−
			1:10	−	−	−	−	−	−
			1:50	−	−	−	−	−	−
		Emulsion of testicle	1:10	−	−	−	−	?	−
			1:100	−	−	−	−	?	−
			1:500	−	−	−	−	−	−
		Fluid medium	1:1	−	−	−	−	−	−
			1:10	−	−	−	−	−	−
			1:50	−	−	−	−	−	−

For legend see Table I.

The strain of vaccine virus employed in the experiments was inoculated once into the brain of a rabbit and afterwards passed several times through rabbits by intratesticular inoculation. Otherwise it was prepared in the same way as in the previous experiments.

The culture medium and the methods are also similar to those of the previous experiments with the exception that testicular tissue was used in the place of the kidney. In some cases the tissue emulsions were inoculated also into the testicle of a living rabbit. The dilutions of each inoculum were prepared with sterile salt solution as follows: culture medium, 1:1, 1:10 and 1:50; tissue, 1:10, 1:100 and 1:500.

The results of the experiments are shown in Tables III to VI.

According to Table III, the spinal cord incubated with the virus twenty-four hours at 37 C. produced visible lesions on the skin earlier than the testicle produced them. In addition to this, the spinal cord diluted 1:100 caused a papule on the skin even after three days' incubation, while the testicle similarly incubated produced no visible lesion even when diluted only 1:10. The activity of both mediums whether containing a piece of spinal cord or testicle was the same. Three days after incubation the virus disappeared from the medium. In other words, as soon as the virus inoculated into the medium containing testicular tissue died out in the medium after incubation, it also disappeared in the tissue. On the contrary, when the virus which was inoculated into the medium containing the spinal cord died out in the medium, it still remained active in the spinal cord even to the following day.

Similar tests for the activity of virus by inoculation into the testicle *in vivo* showed results comparable with those obtained by intracutaneous inoculation as summarized in Table IV, namely, the testicle incubated three days with the virus caused only a very small induration in the testicle while the spinal cord emulsion still produced an intense inflammatory process.

The experiments in which tissues were incubated at room temperature (21 C.), as will be seen in Tables V and VI, gave further evidence of the retention of the virus in the spinal cord. It was noted that the spinal cord incubated with the virus even for twenty-one days was very active and produced a more intense reaction on the skin than when incubated for sixteen days. In other words, the virus in the spinal cord remained very active notwithstanding the

TABLE IV

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at 37 C.)

Rabbit No.	Period of incubation	Inoculum	Size of dose	Gross appearance of reaction at various days after inoculation into testicle					
				4	5	6	7	8	9
31	2 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	–	Slight induration appeared	Induration increased	Induration and swelling	Increasing fullness and firmness	
		Emulsion of testicle	0.2 c.c. (1: 10)	–	Slight induration appeared	Induration increased	Induration and swelling	Induration and swelling slightly increased	
32	3 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration appeared	Induration increased	Marked induration	Fullness and firmness increased	Induration somewhat less than on the previous day	Similar to the previous day
		Emulsion of testicle	0.2 c.c. (1: 10)	–	–	–	Indurated spot appeared	Similar to the previous day	Induration disappearing

long incubation. The virus in the testicular tissue, however, became so weak after twenty-one days' incubation that it produced only a slight inflammatory process on the skin and in the testicle, even with an emulsion diluted 1: 10.

When the spinal cord which was incubated with the virus was inoculated into the testicle of rabbits, as will be seen in Table VI, there took place at the end of two or three days, swelling and induration of the organ, which increased in size and density for five or six days.

TABLE V

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at Room Temperature)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin				
				2	3	4	5	6
38	5 days	Emulsion of spinal cord	1: 10	+	++	++	++	R
			1: 100	+	++	++	++	R
			1: 500	−	+	+	+	R
		Fluid medium	1: 1	+	+	++	R	
			1: 10	+	+	++	R	
			1: 50	−	+	+	R	
		Emulsion of testicle	1: 10	+	+	++	++	R
			1: 100	−	+	++	++	R
			1: 500	−	−	+	+	R
		Fluid medium	1: 1	+	+	++	R	
			1: 10	−	+	++	R	
			1: 50	−	−	+	R	
39	7 days	Emulsion of spinal cord	1: 10	+	++	+++	+++	R
			1: 100	?	++	++	++	R
			1: 500	?	+	+	+	R
		Fluid medium	1: 1	+	+++	+++	R	
			1: 10	+	+	+	R	
			1: 50	?	+	+	R	
		Emulsion of testicle	1: 10	?	++	+++	+++	R
			1: 100	?	?	?	+	R
			1: 500	?	−	−	?	−
		Fluid medium	1: 1	?	++	+++	+++	R
			1: 10	?	+	+	+	R
			1: 50	?	+	+	+	R

For legend see Table I.

TABLE V (*continued*)

Rabbit No.	Period of incubation	Inoculum	Dilution of inoculum	Reaction at various days after inoculation into skin				
				2	3	4	5	6
40	10 days	Emulsion of spinal cord	1:10	++	++	++	++	R
			1:100	−	++	++	++	R
			1:500	−	−	+	+	R
		Fluid medium	1:1	++	+++	+++	+++	R
			1:10	++	++	+++	R	
			1:50	+	+	++	R	
		Emulsion of testicle	1:10	+	++	++	R	
			1:100	−	?	+	R	
			1:500	−	−	+	R	
		Fluid medium	1:1	++	+++	+++	R	
			1:10	+	++	++	R	
			1:50	−	+	+	R	
41	16 days	Emulsion of spinal cord	1:10	−	+	+	+	R
			1:100	−	−	+	+	R
			1:500	−	−	+	+	R
		Fluid medium	1:1	−	−	−	+	R
			1:10	−	−	−	−	−
			1:50	−	−	−	−	−
		Emulsion of testicle	1:10	−	−	+	+	R
			1:100	−	−	?	+	R
			1:500	−	−	?	?	R
		Fluid medium	1:1	−	−	−	+	R
			1:10	−	−	−	−	−
			1:50	−	−	−	−	−
43	21 days	Emulsion of spinal cord	1:10	+	+	++	++	R
			1:100	−	+	+	++	R
			1:500	−	?	+	+	R
		Fluid medium	1:1	+	+	+++	+++	R
			1:10	−	+	++	++	R
			1:50	−	+	+	+	R
		Emulsion of testicle	1:10	?	−	+	+	R
			1:100	−	−	?	+	R
			1:500	−	−	−	−	−
		Fluid medium	1:1	−	+	+	++	R
			1:10	−	?	+	++	R
			1:50	−	−	+	+	R

At this time the color of the testicles was purplish red, spotted here and there with irregular yellowish areas of variable dimensions. In sections many polymorphonuclear leucocytes are seen to invade the interstitial tissues together with a serous exudate; the nuclei of cells within the infiltrated areas become karyorrhectic and some tubules are destroyed. The testicular cells are hydropic and fill up the tubular lumen. The virus in the testicle *in vitro*, however, gradually became attenuated after incubation. In consequence of this attenuation in every instance, the virus gave no intense reaction in the testicle with the exception of that of three days' incubation. The virus after incubating three days in medium containing testicular tissue or spinal cord gave almost the same reactions in the testicle up to six days after the inoculation. At the end of seven days, however, definite differences were noted, namely, the testicle inoculated with the testicular emulsion decreased in size, while that inoculated with spinal cord emulsion still showed an intense reaction. At the end of twelve days the former testicle was normal in size, pale and soft and few small grayish foci were recognized. The sections showed only a small infiltrated area. The latter testicle, however, was covered with a grayish fibrous mass adhering to the tunica vaginalis which was still edematous. The sections showed areas of necrotic tissue and extensive infiltration.

The results obtained in these experiments show that the nervous tissue seems to be endowed with more marked affinity for vaccine virus than the testicle. No marked difference was noted in the effect of the tissue of the spinal cord and that of the testicle on the virus suspended in the medium.

III. Effect of the Spinal Cord on Activity of Vaccine Virus

In view of the results obtained in the foregoing experiments it seems proper to raise the question whether the nervous tissue contains any substances which increase the activity of vaccine virus. To determine this question the following experiments were carried out.

A piece of fresh spinal cord and a piece of the spinal cord previously incubated in the medium (glycerin dextrose broth) for two or three days at 37 C. were separately ground with sterile physiologic salt solution in the proportion of 0.1 gm. of tissue to 0.5 c.c. of salt solution. These tissue emulsions were mixed with the same amounts

TABLE VI

Comparison of the Affinity of Nervous and Testicular Tissues for Vaccine Virus (Incubation at Room Temperature)

Rabbit No.	Period of incubation	Inoculum	Size of dose	Gross appearance of reaction at various days after inoculation into testicle				
				3	4	5	6	7
37 *1	3 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Swelling and induration appeared	Similar to the last	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum	Similar to the last
		Emulsion of testicle	0.2 c.c. (1: 10)	Swelling and induration appeared	Similar to the last	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum	Marked reduction in size
38	5 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration appeared	Similar to the last	Induration increased accompanied by edema of the scrotum	Similar to the last	Marked swelling and edema of the scrotum
		Emulsion of testicle	0.2 c.c. (1: 10)	Slight induration appeared	Similar to the last	Similar to the last	Similar to the last	Induration not increased
40 *2	10 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Swelling and induration appeared	Swelling and induration increased	Marked swelling and induration accompanied by edema of the scrotum		
		Emulsion of testicle	0.2 c.c. (1: 10)	Swelling and induration appeared	No increase	No increase		
43 *3	21 days	Emulsion of spinal cord	0.2 c.c. (1: 10)	Slight induration	Marked induration	Marked swelling and edema of the scrotum		
		Emulsion of testicle	0.2 c.c. (1: 10)	No sign	Slight induration	Similar to the last		

*1 Twelve days after inoculation both testicles were removed. *2 and *3 Five days after inoculation both testicles were removed.
All these testicles were fixed in Zenker's fluid and sections cut in paraffin.

of a highly virulent virus (original mixture diluted 1:100); these mixtures were further diluted 1:10 with sterile salt solution. Then 0.1 c.c. of each mixture was inoculated into the skin of a normal rabbit. As control, the virus and the emulsion of spinal cord without vaccine virus similarly diluted with sterile physiologic salt solution, were inoculated.

The results of these experiments are shown in Table VII.

TABLE VII

Effect of the Spinal Cord on Vaccine Virus

Rabbit No.	Spinal cord and control	Inoculum	Dilution of inoculum	Size of dose	Reaction at various days after inoculation into skin				
					3	4	5	6	7
62	Fresh	Vaccine virus with spinal cord	1:1 (original)	0.1 c.c.	+	+	+	+	R
			1:10	0.1 c.c.	−	+	+	+	R
	Control	Vaccine virus without spinal cord	1:1 (original)	0.1 c.c.	−	−	+	+	R
			1:10	0.1 c.c.	−	−	+	+	R
		Emulsion of spinal cord	1:1	0.1 c.c.	−	−	−	−	−
63	2 days incubated	Vaccine virus with spinal cord	1:1 (original)	0.1 c.c.	+	+	+	R	
			1:10	0.1 c.c.	+	+	+	R	
	Control	Vaccine virus without spinal cord	1:1 (original)	0.1 c.c.	−	+	+	R	
			1:10	0.1 c.c.	−	+	+	R	
		Emulsion of spinal cord	1:1	0.1 c.c.	−	−	−	−	−
64	3 days incubated	Vaccine virus with spinal cord	1:1 (original)	0.1 c.c.	−	−	+	+	++
			1:10	0.1 c.c.	−	−	−	−	+
	Control	Vaccine virus without spinal cord	1:1 (original)	0.1 c.c.	−	−	+	+	+
			1:10	0.1 c.c.	−	−	−	−	?
		Emulsion of spinal cord	1:1	0.1 c.c.	−	−	−	−	−

For legend see Table I.

The results show that the virus inoculated with the emulsion of spinal cord caused stronger inflammatory processes in the skin of a normal rabbit than the virus without spinal cord. In view of this fact, there seems no doubt that the spinal cord of a normal rabbit has a suitable substance for promoting the activity of vaccine virus.

IV. Is Vaccine Virus Capable of Migrating along the Spinal Cord *in Vitro?*

To determine this question the following experiments were carried out. A piece of the spinal cord about 6 cm. long was placed straight in a Petri dish. A mixture of 15 per cent agar (one part) and glycerin dextrose broth (one part) was poured into the dish until the piece of spinal cord was totally covered. After the poured mixture hardened, one end of the piece of spinal cord was exposed by removing a little agar, and at this end a small drop of vaccine virus was inoculated. The dish was placed at room temperature (21 C.). After incubation for different periods of time 0.1 cm. was cut off each end of the spinal cord. These end-parts of tissue were separately emulsified by grinding with sterile salt solution in the proportion of 0.1 gm. of tissue to 0.5 c.c. of salt solution. A normal animal was inoculated with 0.2 to 0.3 c.c. of the emulsions and also with the agar surrounding the uninoculated end of the cord.

TABLE VIII

The Question of Migration of Vaccine Virus along Spinal Cord in Vitro

Rabbit No.	Period of incubation	Inoculum	Method of inoculation	Result
54	3 days	1	Intradermal	+++
		2	"	–
		3	"	?
58 *	3 days	2	Intratesticular	?
		3	Intradermal	?
59	4 days	1	Intradermal	+++
		2	"	–
		3	"	–

1 = emulsion of tissue of inoculated end.
2 = emulsion of tissue of uninoculated end.
3 = agar surrounding uninoculated end.
* Two weeks after first inoculation revaccination was made into the skin but no response was obtained.

The results of these experiments, as summarized in Table VIII, show that the tissue emulsions of the uninoculated end failed to produce a visible lesion, while in two cases agar surrounding the tissue caused a doubtful reaction on the skin. In view of these facts it is evident that vaccine virus is incapable of migrating along the spinal cord *in vitro*, although there is a possibility that it may be transmitted through a space between the spinal cord and agar (capillary attraction?).

Discussion

According to the embryonic origin of tissues the spinal cord is an invaginated segment of the ectoderm, so that it can be inferred that the central nervous system of the rabbit affords as favorable a site for the multiplication of the virus as the skin. But when the virus is injected into the blood stream, the brain is either deprived or fairly free of vaccine virus. In this connection Levaditi [4] has announced that it is probable that the vascular endothelium (or the entire choroid plexus) prevents the passing of vaccine virus from the blood stream into the nervous system. On the other hand, he produced evidence that the virus grows abundantly in the brain when normal saline or broth is injected into the brain of an intravenously infected animal, because the irritation due to the intracerebral injection of saline or broth causes an aseptic meningitis and breaks down this resistance, thus offering to the virus an excellent culture medium, the brain itself.

My experiments *in vitro* show that the central nervous system of the rabbit is endowed with more marked affinity for the vaccine virus than the testicle. Furthermore it seems as if the virulence of the virus increases in the nervous tissue to some extent. Therefore it is probable that of all tissues the central nervous system contains the most favorable substances and soil for propagation of the virus although the virus does not multiply in the nervous tissue removed from the body.

Steinhardt and his co-workers [7, 8] applied the method of cultivating corneal tissues *in vitro* to the study of vaccine virus and stated that there was slight multiplication of the virus. Recently Parker [9] announced that he had succeeded in carrying vaccine virus through many generations in artificially cultivated testicular tissues. If the virus is really able to grow in tissue cultures, it would be expected

that the central nervous system, being endowed with a specific affinity for the virus, should be the favorable tissue for such a purpose. Experiments to decide this point are now under way.

Summary

1. Vaccine virus has an affinity for the central nervous system but almost no affinity for the kidney *in vitro*, the latter indeed having a deleterious effect on the virus.

2. Vaccine virus has also an affinity for testicular tissue but this is less marked than its affinity for nervous tissue.

3. Vaccine virus survives in nervous tissue immersed in fluid medium after dying out in the latter.

4. Vaccine virus in culture medium containing spinal cord produces a more marked reaction on the skin of rabbits than the virus in medium containing kidney, although the difference is by no means sharp; however, there appears to be almost no difference in the effect of spinal cord and testicle on the virus suspended in the medium.

5. The vaccine virus emulsified with spinal cord causes more marked inflammatory reaction than control glycerinated virus. Therefore, it would appear that the spinal cord of a normal rabbit is favorable to the growth of the virus and tends to increase its virulence.

6. Vaccine virus appears to be incapable of migrating along the spinal cord *in vitro*.

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SPONTANEOUS CENTRAL NERVOUS SYSTEM LESIONS IN THE LABORATORY RABBIT *

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Recently, various investigators have reported a spontaneous encephalitis in experimental rabbits which has often proved very misleading, especially when the rabbit is used in experiments on the central nervous system. The reports are widely distributed, coming from Twort [1, 2] in England, Levaditi [3] in France, Plaut [4] and Pette [5] in Germany and Doerr [6] in Switzerland. The earliest report was by Bull [7] in the United States. Levaditi,[3] Wright and Craighead [8] and Oliver [9, 10] claim to have found an associated organism which Levaditi calls Encephalitizoön cuniculi. Other reports have been made by Flexner,[11] Wright [8] and Goodpasture and Teague.[13]

These lesions particularly concern us in our laboratories as our work is largely with the central nervous system and several series of experiments had been planned with the rabbit. A careful examination of the brains of all rabbits used during the year of 1924 was therefore made to determine the frequency of the lesions in our stock.

Forty rabbits were used during this year. Twenty-four were used in experiments with insulin, twenty receiving injections to the point of convulsions and death and four being untreated control rabbits. Six rabbits were used in experiments with nerve degeneration and regeneration. Seven rabbits were used in experiments with cholesterol injections. Three young rabbits were born during the course of the experiment and were untreated.

This group of animals included both males and females of all ages from one month to adults weighing nearly 4000 gm. Some had been pregnant and had delivered their young; others were pregnant at the time of death. Clinically some of the rabbits had had snuffles, convulsions and pneumonia. Postmortem evidence of pneumonia, otitis media and liver parasites was found. No relation existed between the experiments performed and the lesions in the central nervous system, although the insulin group showed the least number of lesions of the severe grade. Some relation seemed to exist between

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infections of the upper respiratory tract and the lesions, in that all rabbits showing any marked respiratory symptoms had at least a two plus meningeal reaction, focal lesions or both. However, the rabbit which suffered the most marked clinical symptoms of snuffles had only a three plus meningitis and no focal lesions, while the rabbit showing the most marked histologic picture of severe focal and meningeal lesions had not shown any symptoms.

The meningeal reactions found in the rabbits of our laboratories have been classified as follows: negative reaction in which the brain and meninges do not show definite lesions of this type; one plus in which there is a very slight increase in the lymphocytic cells of the meninges with dilated capillaries throughout the brain, especially of the agranular layer, with slight increase of the cells around these vessels; two plus, the same but more intense; three plus, a marked proliferative meningitis and perivasculitis; and four plus in which there are focal lesions in the brain substance which are always associated with a two and three plus meningitis and perivasculitis.

The number of rabbits having these different conditions were:

Negative	3	rabbits
One plus	20	"
Two plus	7	"
Three plus	1	"
Four plus	9	"
Total	40	"

Associated pathologic findings. The liver usually shows a proliferation of lymphocytes about the triads. The kidneys also have areas of lymphocytes in the interstitial tissue with an occasional small granuloma like the focal lesions in the brain. Numerous eosinophiles are in the spleen.

Description of the Lesions in the Central Nervous System. In the meninges there is an increase in the cells of the subarachnoid space and beneath the pia. These cells are usually small without much cytoplasm. In the more severe cases epithelioid and plasma-like cells with eosinophilic cytoplasm occur. This reaction is most marked over the cerebrum, though it frequently is found about the cerebellum and over the medulla when focal lesions are present there. It is not seen to any marked degree in the spinal cord. The largest accumulation of cells occurs about the points of entrance of vessels into the brain substance.

In addition to the meningeal reaction, the small blood vessels which extend into the cerebrum through the agranular layer are usually dilated and there is a slight increase in the cells in the walls of these vessels. In the mild cases, this cellular exudate consists of cells having small dark nuclei. Half of the rabbits furnished at least this much of a reaction and indeed it was difficult to find a rabbit which could be considered entirely free from some cellular reaction. In the more severe cases, the capillaries throughout the cerebral tissue are dilated and the perivascular proliferation is productive in nature, there being more cells with cytoplasm which is often eosinophilic. The reaction is always most pronounced in the vessels which extend in from the meninges.

The focal lesions are most frequently found deep in the cortex. None is seen in the cerebellum. Several are found in the gray matter of the medulla and midbrain. A few are found in the cord both in the white and gray matter. Careful search will usually show that these are as a rule associated with small vessels which extend in from the meninges. The vessels in question show a perivascular proliferation throughout their course and the lesion appears at the tip like a flower on a stalk.

The lesions are distinctly granulomatous in nature. The center shows a small mass of cell detritus surrounded by cells with irregular nuclei and considerable cytoplasm, the limits of which cannot be readily distinguished. About these are small cells with small dark nuclei and spindle-shaped bodies which often run out into tails. At the periphery of the granuloma in the more severe cases there occur definite spider cells which penetrate some distance into the brain tissue as do the small round cells. Around such a lesion occur numerous smaller ones consisting of round cells lying about the adjacent capillaries.

In a few instances, minute dark staining bodies about two to three microns in diameter have been seen in and about blood vessels, associated with some of the most severe lesions. These may be the bodies described by Levaditi, Wright and Craighead and Oliver as the Encephalitizoön cuniculi. Jahnel's spirochete stain failed to demonstrate any spirochetes.

A phosphotungstic acid hematoxylin preparation shows some, though not many, glia cells which have formed fibrils. Giant, true plasma and polymorphonuclear cells are not seen.

A reaction about the ependyma of the ventricles similar to that associated with the meninges has been described by Oliver and others. But a developmental heaping up of cells in the ependyma of rabbits is frequent and is easily confused with such a condition.

Conclusion

It is quite clear that lesions which are so extensive and occur so frequently render the rabbit unfit for most experiments on the central nervous system.

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EXPERIMENTAL PRODUCTION OF GLIOSIS *

I. EFFECTS ON THE NERVOUS SYSTEM OF THE RABBIT OF INTRAVENOUS AND INTRASPINAL INJECTIONS OF CHOLESTEROL EMULSION

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A multiplication of glia cells (gliosis) is one of the common reactions of the nervous system to lesions. The reaction is similar to mesodermal scar formation in lesions of other parts of the body. The exact cause of this reaction has not been determined. It may be a purely mechanical replacement of lost tissue; it may be that toxins or other noxious agents which injure or destroy parenchymatous tissue act as a stimulus to the reproduction of the less specialized supportive tissues; or it may be that products of degeneration or the injured nerve tissue act as a specific stimulus to the glia cells. Quite commonly, vascular accidents in the brain destroy fibers of the pyramidal tract in the internal capsule, thus causing a degeneration of these fibers through the midbrain, medulla and cord; and this long tract, far removed from the original lesion, develops a characteristic glia scar throughout its course as a reactive rather than inflammatory process. It was with this idea in mind that a series of experiments were planned in which extracts of brain tissue were to be introduced into the nervous system of animals and the reactions of the glia cells studied.

In this particular experiment, cholesterol was used because it is a lipoid present in the brain in large quantities. Various workers[1] have determined the percentage of cholesterol to be from 1 to 4 per cent in different parts of the fresh human brain. In order to maintain a constant supply of the cholesterol and thus to attempt to produce a chronic condition, the cholesterol was introduced at frequent intervals intravenously and by lumbar puncture.

Merck's purified cholesterol was used in a 1 per cent emulsion. The method of the preparation of the emulsion was somewhat similar to that used by Kaethe Dewey,[2] who recommended pouring an

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acetone solution of the cholesterol into hot water, concentrating as desired and filtering. In our hands, this preparation salted out when sodium chloride was added to obtain a physiologic salt content, or when salt solution, blood serum or whole blood was added. It was found, however, that if an acetone or hot alcoholic solution of cholesterol was poured into a boiling weak saline solution of about one-fourth the strength of a physiologic solution, this emulsion could be concentrated until it was of physiologic strength in its saline content. It was, however, a little stronger than desired in its cholesterol content and could then be diluted with ordinary physiologic saline solution. This emulsion did not break down with time, by boiling or by the addition of salt solution, water or blood serum. Control fluids were made by adding the same amount of alcohol to the weak saline solution and treating this as the emulsion was treated. There was no evidence of any alcohol remaining in any of the preparations.

For intravenous injection, 5 c.c. of a 1 per cent cholesterol emulsion was injected daily into the large vein of a rabbit's ear. One rabbit received twenty injections, a second thirty injections and a third forty injections. A fourth rabbit received twenty-six injections of the control fluid. For the intraspinal injections, cholesterol emulsion of a 1.5 per cent strength was used. Lumbar punctures were made on the rabbits at about weekly intervals, as much spinal fluid being removed as could be obtained and about 1 c.c. of the emulsion introduced at each time. Four rabbits were injected with the emulsion and one with the control fluid.

Careful necropsies were performed and tissue samples taken from brain, spinal cord, liver, kidney and other viscera. Sections after Zenker's fixative stained with eosin-methylene blue were prepared to determine the routine histologic picture. Scharlach R stains of frozen sections of formalin-fixed material gave good results for the detection of cholesterol. Hurst[3] points out that pure cholesterol does not stain with Scharlach R, but stains only if mixed with fatty acids or neutral fats. However, as the bodies here interpreted as cholesterol were found to take the Ciaccio stain as well as to be doubly refractile, it must be assumed that the cholesterol has taken up some fats or that the pure cholesterol is staining in these cells. In addition, Ciaccio's method (Bell[4]) for staining intracellular lipoids was used and found quite as delicate for demonstrating

cholesterol as the usual Scharlach R method. The cholesterol is apparently not lost in any appreciable amounts in the dehydrating agents. It was assumed by Ciaccio that the prolonged chromation rendered the lipoids subject to staining by Scharlach R while the dehydrating process dissolved out the fats and fatty acids. However, there is some question whether chromation does not make fats less soluble to the ordinary fat solvents. The polarizing microscope was used to identify the cholesterol by its doubly refractile character. For the study of the glia cells, Mallory's phosphotungstic acid hematoxylin and Bailey's [5] neutral ethyl violet-orange G method were used.

Protocols of the experiments with the intravenous injections are as follows:

B C. Control. Twenty-six injections of 5 c.c. each of the control fluid were given intravenously over a period of forty-six days. The Ciaccio stain of the liver shows a few cells about the triad containing a definite though slight amount of lipoid substance. In the kidney there is a tendency toward lipoid staining in the interstitial tissues of the pyramids. The Ciaccio stain shows no lipoid substance in the meninges of the spinal cord. In the brain a few round cells occur in the perivascular spaces and a four plus encephalitis of the spontaneous type is present. This is the condition described in the first paper of this series and where it was encountered it was assumed not to be a result of the experiment. By the Ciaccio stain, lipoids are shown in large distinct globules in some of the cells of these areas. The polarizing microscope reveals doubly refractile bodies normally present in the spinal cord and brain and a scattering of doubly refractile bodies in the lobules of the liver. The significant findings in this control animal are: (1) some peribiliary cells containing a slight amount of lipoid which stain by the Ciaccio method, showing that it is not soluble in alcohol or chloroform after the chromating process; (2) lipoids in the interstitial tissues of the kidney; and (3) lipoids in the cells reacting to the spontaneous meningitis.

B I. A male rabbit received twenty doses of 5 c.c. each of a 1 per cent emulsion of cholesterol, totaling 1 gm. of the lipoid in twenty days. A considerable amount of substance staining with Scharlach R is seen about the triads and in the Kupffer cells in the Ciaccio preparations. The kidney contains areas of interstitial lymphocytes and nodular areas like the encephalitic focal lesions. Lipoid in the inter-

stitial tissues is present as in the control. The brain shows a four plus encephalitis. No lipoid is found anywhere in the brain and there is no evidence of an increase in glia cells. The significant findings in this rabbit are: (1) lack of evidence of cholesterol anywhere except in the liver; (2) absence of gall stones, sclerosis of the aorta, endothelial ingestion of cholesterol or any of the other results of hypercholesterolemia frequently reported in the literature; and (3) absence of any glial reaction to the cholesterol.

B III. A female rabbit received thirty 5 c.c. doses of a 1 per cent emulsion of cholesterol, amounting to 1.5 gm. in thirty-two days. It was killed eleven days after the last injection and was found to be pregnant. Considerable amounts of lipoid material are seen in the mesodermal cells of the liver in the Ciaccio preparations. In the kidney, lipoids are present in the interstitial tissues in the pyramids and extending into the medulla and in the convoluted tubules. Considerable fatty changes have occurred in the renal and hepatic cells. The brain is the seat of a four plus encephalitis. A marginal gliosis has developed and a few cells in the perivascular spaces contain a slight amount of lipoid. The erythrocytes tend to take the lipoid stain. The significant findings are: (1) cholesterol in the mesodermal cells of the liver and also in the hepatic and renal cells; (2) a slight amount of lipoid in the mesodermal cells of the brain which may be normal; and (3) no definite evidence of a noteworthy increase in glia cells of the brain and spinal cord.

B II. A female rabbit received forty doses of 5 c.c. each of a 1 per cent emulsion of cholesterol, totaling 2 gm. given over a period of sixty days. She delivered young, developed snuffles and lost considerable weight. In the Ciaccio preparations of the liver are seen lipoids in the Kupffer cells and cells of the triads. There is about twice as much as in B I, which received half as much cholesterol. In the brain is a two plus meningitis and lipoids occur in some of the cells of this reaction. Doubly refractile bodies of the tissues are normal except for an increase in the liver. Significant findings are: (1) evidence of cholesterol deposits in the liver and kidney in amounts directly proportional to the amount given; (2) a possibility that cholesterol has been deposited in the meningeal and perivascular cells of the encephalitic reaction in the brain as a result of the injection.

Final Summary of Results by Intravenous Injection

When cholesterol was injected intravenously it was deposited in increased amounts in the mesodermal cells of the liver in proportion to the amount given, although the rabbit receiving none showed a slight amount. It may be deposited in increased amounts in the pathologic cells (mesodermal) of the meninges and perivascular spaces in spontaneous encephalitis. The control animal showed more than the one receiving 1 gm. but less than those receiving 1.5 gm. and 2 gm.

TABLE 1

Results Obtained by Intravenous Injection of Cholesterol

Rabbit number	Total cholesterol injected	Brain			Liver		Kidney	
		Meningeal lipoids	Perivascular lipoids	Glial reaction	Mesodermal lipoids	Hepatic lipoids	Interstitial lipoids	Renal lipoids
	gm.							
B C	0	+	?	−	+	+	++	+
B I	1	−	−	−	++	+	++	+
B III	1.5	±	+	−	+++	++	+++	+++
B II	2	++	++	−	+++	+	++	+

B III, although not receiving as much as B II, showed more marked deposits of cholesterol. This may have been due to pregnancy or to the fact that the encephalitis was more severe in this animal. The fact that the erythrocytes stained more brightly may be evidence of a greater lipoid accumulation in the tissues of this animal or may indicate that for some reason the lipoids in this animal have stained more readily than usual. Lipoids are normally present in the interstitial tissues of the pyramids of the kidney but they may be increased in the hypercholesterolemia, and while those normally present are not doubly refractile, with the intravenous injection of cholesterol refractile bodies tend to occur. No evidence of any sclerosis of the aorta or of gall stones was found, nor of any increase in cholesterol in any of the tissue elements of the nervous system or of any glia or nerve-cell reaction to the hypercholesterolemia.

Discussion

Several investigators have produced experimental hypercholesterolemia and have reported changes such as sclerosis of the aorta, formation of gall stones and hepatic and renal lesions in animals some of which had received no more cholesterol than the rabbits of this series. In Dewey's experiments, three rabbits which had received 0.2 gm., 2 gm. and 2.4 gm. intravenously, showed all of these lesions in one or more of the animals. Bailey [6] produced sclerosis of the aorta by feeding cholesterol or egg yolk. But it is hard to say how this compares with intravenous injections of pure cholesterol.

TABLE 2

Results Obtained by Intraspinal Injection of Cholesterol

Rabbit number	Total cholesterol injected	Lumbar punctures	Death after last lumbar puncture	Thickening of meninges	Lipoid in meninges	Gliosis in cord
	gm.					
S C	0.000	2 in 2 weeks	7 days	–	–	–
S I	0.045	3 " 2½ "	9 "	+++	+++	–
S II	0.045	3 " 2½ "	7 "	+++	+++	+
S III	0.060	4 " 5 "	2 "	++	++	+
S IV	0.075	5 " 6 "	35 "	–	–	+

Tunnicliff [7] reports that cholesterol in small quantities increases phagocytosis both *in vivo* and *in vitro* but in larger quantities it decreases it. Similar results were obtained by Dewey and Nuzum.[8] This may have been a factor in the increased content of lipoid substance in the cells of the meningeal and perivascular reaction in the encephalitis of our animals. However, this work only definitely demonstrates an ingestion of the cholesterol by the mesodermal cells of the liver and possibly by the reactive cells in the brain which are present as a result of this spontaneous encephalitis. There is also a possibility that the lipoid content of the liver and kidney cells and of the erythrocytes is increased. These very moderate results were obtained in spite of the fact that two of the rabbits were pregnant, which is supposed to increase the lesions produced by the hypercholesterolemia.

As far as known, there has been no previous work on the effects of a hypercholesterolemia on nerve tissue. Parhon and Parhon [9] re-

port an increase in cholesterol in the blood in chronic alcoholism, senile dementia and some paralyses, while there is a decrease in dementia and melancholia. Pighini [10] records finding cholesterol in the spinal fluid in 68 per cent of the cases of paresis, often in epilepsy and in dementia praecox but none in the other conditions. Weston [11] reports that cholesterol can be found in the cerebrospinal fluid in all cases of mental disease, the amount having no relation to the type of the psychosis.

With the hope of bringing the cholesterol into close contact with the nerve tissue, intraspinal injections were made in rabbits. Lumbar punctures were done about once a week and 1 c.c. of a 1.5 per cent emulsion in physiologic saline was introduced after spinal fluid was removed.

Protocols of these experiments are as follows:

S C. A female rabbit received two intraspinal injections of the control fluid with an interval of two weeks between. Death followed one week after the last injection. The meninges of the cord are normal. No unusual lipoids are seen by the Ciaccio stain.

S I. A male rabbit received 3 c.c. of a 1.5 per cent cholesterol emulsion in three intraspinal injections in two and a half weeks and died nine days after the last and twenty-five days after the first injection. It received 0.045 gm. of cholesterol intraspinally.

The dura and arachnoid membranes of the cord are very much thickened, especially over the posterior surfaces. In the dura the thickening is fibrous but in the arachnoid it consists of large vesicular cells. In the Ciaccio preparations these cells are filled with lipoids. The Scharlach R preparations show the same picture very well. Kubie and Schultz [12] claim that the arachnoid cells are non-phagocytic and that phagocytes in the meninges are special cells which multiply rapidly in response to irritation. In any case, in these experiments there is a very striking increase in the large phagocytic cells in the arachnoid space. Bailey's stain for neuroglia fibrils shows a normal glia content in the cord. The polarizing microscope demonstrates doubly refractile lipoids in the cells of the arachnoid space. The significant findings are: (1) a fibrous increase in the dura and a cellular increase in the arachnoid, in which the cholesterol is found; and (2) no glia or nerve-cell reaction.

S II. A male rabbit received 3 c.c. of a 1.5 per cent cholesterol emulsion or 0.045 gm. of cholesterol by three intraspinal injections

over a period of two and one half weeks. Death occurred seven days after the last injection. The spinal cord shows a cellular increase in the arachnoid and axonal chromatolysis of some of the anterior horn cells. The Ciaccio and Scharlach R preparations demonstrate lipoid in considerable quantities in cells of the arachnoid. In an area of trauma are extravasated erythrocytes and an increase in the glia fibrils. A slight increase in the glia cells is present but may be associated with the trauma. The polarizing microscope reveals doubly refractile droplets within the arachnoid cells. The significant finding is an increase in the arachnoid cells which ingested the cholesterol.

S III. A female rabbit received 4 c.c. of the emulsion or 0.06 gm. of cholesterol by four intraspinal injections in six weeks. She was killed one week after the last injection and found to be pregnant. The significant findings are: (1) an increase in cells about the area of trauma and the vessels and in the meninges, with an ingestion of cholesterol; and (2) a moderate glia reaction in the cord.

S IV. A female rabbit received 5 c.c. of a 1.5 per cent cholesterol emulsion or 0.075 gm. in five intraspinal injections during a period of six weeks. She delivered young during this time and was in a poor condition when killed eleven weeks after the first, and five weeks after the last injection. The meninges of the spinal cord are at no place thickened beyond the normal limits nor has any increase in the cellular contents of the arachnoid occurred. The Ciaccio preparations give no evidence of an abnormal lipoid anywhere in the meninges or elements of the cord. Bailey's stain shows a moderate increase in the glia cells of the periphery of the cord adjacent to the large deposits of cholesterol in the meninges. No unusual amount of doubly refractile bodies can be seen with the polarizing microscope. The significant findings are: (1) a moderate gliosis of the spinal cord; and (2) no increase in the dura or arachnoid and no cholesterol deposits at the site of the injection in the cord. On the whole the results were surprisingly negative considering the amount of cholesterol injected. This rabbit received a good deal more than any other and spinal fluid removed a week to ten days after the injection was found on several occasions to develop cholesterol crystals when allowed to evaporate. However, five weeks had elapsed between the last injection and the time of death, during which period the extraneous cholesterol had apparently been removed. The meningeal reaction, which presumably was as great in this animal

as in the others, must also have disappeared leaving only a moderate gliosis.

Conclusions

The conclusions from this series of experiments as far as the nerve tissues are concerned are that a hypercholesterolemia in the relatively small amounts used here has no effect on the cells of the nervous system except possibly to increase the lipoid content of the phagocytic cells of the meninges or perivascular spaces, or in focal lesions already present due to other causes. Introducing the cholesterol directly into the subdural space of the spinal cord causes a thickening of the dura and a marked increase in cells of the arachnoid space which are phagocytic and ingest the cholesterol, and a moderate increase in the glia cells of the periphery of the cord. Five weeks after the last injection the meningeal and arachnoidal reaction is not seen, though the gliosis persists.

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EXPERIMENTAL PRODUCTION OF GLIOSIS *

II. REACTION OF BRAIN TISSUE TO THE LIPOID FRACTIONS AND TO THE RESIDUE OF BRAIN EXTRACTS

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The striking difference in the chemical structure of brain and spinal cord tissue and the other tissues of the body lies in the high lipin content of the brain and cord. These lipins do not include the neutral fats but consist almost entirely of the more highly complex lipoids, the most important of which are cholesterol, phospholipin and glycolipin or cerebroside. These lipoids are important constituents in all of the cells of the body and their large proportion in the nervous tissue is not due to a cellular increase but to the fact that they are the principal constituents of the medullary sheaths. This is brought out by a comparison of analysis of brain tissue taken from the white matter such as the corpus callosum and the gray matter or child's brain in which medullation is still incomplete.

It would seem possible that one or all of these lipoids might be an important factor in the production of gliosis in the degeneration of the long fiber tracts, such as the pyramidal tract, when no inflammatory process is present to provide stimuli for the multiplication of the glia cells. It is our intention, therefore, to extract brain tissue and obtain these lipins and the residue and to determine the effect of these substances upon normal brain tissue.

Dogs were given ether anesthesia, the femoral vein cannulated, the femoral artery on the same side opened and the blood washed out of the animal with physiologic saline as long as the heart continued to beat. As soon as the cardiac action ceased, the common carotid arteries were cannulated, the jugular veins opened and washing with physiologic saline by gravity was continued until the returns were clear. The brains were removed, stripped of all meninges and superficial vessels and delivered to the chemical laboratory of the hospital, to be extracted by the method given in Mathews' Physiological Chemistry.

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A precipitate is obtained by cooling a hot alcoholic extract. The ether soluble substances are then removed and the remainder consists chiefly of the cerebrosides. To the ether extract thus removed is added the cold alcoholic filtrate; acetone soluble substances are removed and the remainder is chiefly phospholipins. The acetone extract from the above is chiefly cholesterol. The residue from these extractives contains the nucleo- or phosphoproteins. The first three

TABLE I

Composition of the Brain. Thudichum[1]

(Corrected for dried tissue)

	Corpus callosum	Gray matter
	per cent	*per cent*
Neuroplastin	30.0	53.0
Ether extract (Phospholipin and cholesterol)	38.0	18.5
Cerebrosides and myelin	23.0	2.5
Water extracts	4.7	3.3

White matter exceeds gray matter in phospholipins and cholesterol by 38 to 18.5.
White matter exceeds gray matter in cerebrosides by 23 to 2.5.

Composition of the Brain. Koch[1]

	Corpus callosum	Child's brain
	per cent	*per cent*
Protein	27.0	46.0
Extractives	4.0	12.0
Cerebrosides	18.0	7.0
Phosphatides	31.0	24.0
Cholesterol	17.0	1.8

White matter exceeds child's brain in phosphatides and cholesterol by 48 to 26.
White matter exceeds child's brain in cerebrosides by 18 to 7.

may be considered to represent roughly the materials chiefly derived from the medullary sheaths, and the fourth those from the cells.

These substances were prepared in a sterile condition by evaporating the cerebrin out of methyl alcohol, the phospholipin out of acetone, the residue out of ether and by heating the cholesterol to 120 C. for two hours. These were then preserved in sealed tubes.

Trephine openings were made over the parietal area in dogs under ether anesthesia, the meninges snipped with the scissors, a hole

made in the brain with a small trocar and a piece of the extract pushed through the hole. Five dogs were operated on, one serving as a control while each of the others received one of the extracts. The dogs were allowed to live four weeks. Pieces of the injected area were fixed in formalin, Zenker's solution and Ciaccio's solution. They were stained with Scharlach R, Ciaccio's stain, eosin-methylene blue, iron hematoxylin, phosphotungstic acid hematoxylin, neutral ethyl violet-orange G and the aniline blue connective tissue stain. The material was also studied with the polarizing microscope.

Control. No evidence of infection found. The meninges are moderately thickened at the point of incision. The hole in the brain can be readily seen.

A section through the area of the lesion shows moderate hemorrhage with phagocytes containing blood pigment. The blood vessels in and adjacent to the lesion are prominent. The endothelial cells are slightly increased in the perivascular spaces and the mesodermal fibrous tissue is slightly increased within the area as shown by the aniline blue connective tissue stain. A moderate gliosis of the spider cell type and a few cytoplastic glia cells have appeared adjacent to the lesion.

The significant reactions to this lesion were an increase in endothelial cells and fibroblasts of the blood vessels with some invasion of the area of the lesion, phagocytes full of blood cells and débris, and a moderate gliosis.

Phospholipin. The phospholipins are contained in the ether extract of the brain tissue. They are one of the major constituents of the so-called protagon of the medullary sheaths and also of all cellular protoplasm. They are characterized by Mathews [1] as being a "water-containing, semifluid, highly-reducing, auto-oxidizable, semilipoid crystalline substratum, which contributes to or makes possible the vital phenomena." They are also important in immunity and it is suggested that they are an important factor in staining of the living cell by basic dyes. They constitute 31 per cent of the dried white matter (Koch) and may thus be an important factor in producing gliosis in tract degeneration.

The important phospholipins of the brain are lecithin, cephalin, myelin, paramyelin and sphingomyelin. The chief difference in these substances is apparently in the fatty acid radicle. Lecithin has been the most studied and it is the only one whose formula is

known. On hydrolysis it yields glycerine, oleic acid, stearic acid, phosphoric acid and choline. Thudichum[1] claims that the chief properties of lecithin are due to its oleic acid. For our purposes, however, the fatty acids may prove of less interest than the phosphoric acid and choline.

Choline is a nitrogen base and has powerful physiologic action. By intravenous injections of minute quantities it increases the blood pressure (Mendel et al.[2]) and by intracerebral injection it produces convulsions and paralysis (Donath[3]). Coriat[4] speaks of it as "the most important decomposition product of lecithin which is found in the central nervous system, blood and cerebrospinal fluid in conditions where active degeneration is taking place." He claims that it is found in cerebrospinal fluid, brain and cord only with active medullary sheath degeneration which leads to decomposition of the lecithin and to a coincident increase in phosphoric acid in cerebrospinal fluid. Donath[5] claims that choline and glycero-phosphoric acid pass into the fluid on decomposition of lecithin while stearic acid combines with glycerol to form neutral fats which accumulate beneath the neurolemma.

The extract used in this work must be considered an impure mixture of the various phospholipins and specific results are not to be expected.

The trephine opening is filled with fibrous tissue and the meninges are adherent to it and the underlying brain at the point of the lesion. The meninges are thickened at this point. A vessel in the meninges apparently draining this area is very conspicuous by its size and the thickness of its wall.

On cutting the sections, the microtome knife met with considerable resistance at the point of the lesion. The knife was nicked and the tissue torn. This was apparently due to calcification of the injected phospholipin occurring within the four weeks during which it had been in the brain. Among the various theories of pathologic calcification, the best accepted is the formation of calcium-binding substances within degenerating areas, especially phosphoric acid and fatty acids (Wells[6]). It has been suggested that calcium soaps are formed from the fatty acids and are then transformed into the less soluble phosphates and carbonates. Cellular lecithin and nucleoproteins have been considered as the probable sources of phosphoric acid and fatty degeneration has been recognized as a

precursor of calcification. However, Wells questions these theories. In a chemical study of a large quantity of material he found only traces of calcium soaps in calcifying matter even in the early stages (Wells [7]). He has implanted in the abdominal cavity of rabbits various tissues that had been killed and sterilized by boiling and found that the tissues rich in nucleoproteins show no greater tendency to calcify than those poor in nucleoproteins. However, the residue used by the writer, which presumably consisted largely of nucleoproteins, also produced substances in the brain giving marked resistance to the microtome knife, though it was not nicked. None of the lesions produced by the other extracts seemed unusually hard. This seems to suggest that the phosphoric acid may play some part in calcification. The phospholipins, however, contain both phosphoric acid and the fatty acids.

Studies of the lesion produced by the phospholipin show a picture very similar to that of the control as far as the mesodermal elements are concerned. Hemorrhagic elements are present with phagocytes containing blood pigment. The blood vessels in and about the lesion are prominent because of a slight endothelial and fibroblastic increase. The area contains some fibrous tissue. However, more phagocytes occur here than in the control lesion and many of these contain a substance stained by Scharlach R. Gliosis of the fibril-forming type is more marked but still moderate.

It would appear then that except for the calcification, the hypertrophy of the meningeal vessels and the greater gliosis, the effect of introducing phospholipins into the brain was no greater than the mechanical effect of the trocar hole as seen in the control animal. Possibly if this extract were further broken down into its constituents, namely, pure lecithin or choline, phosphoric acids and the fatty acids, a more pronounced reaction would be obtained.

Cerebrin. The cerebrosides are glycolipins and phosphorus free. They are obtained from the hot alcoholic extract. On hydrolysis they yield a sugar, usually galactose, various fatty acids and a nitrogen-containing alcohol. The common ones found in the brain are phrenosin, kerasin and cerebron. They are not present in the embryonic brain but increase with medullation and constitute 18 per cent of the dried adult corpus callosum (Koch). Apparently less work has been done with the cerebrosides than with the phospholipins from either the chemical or the biologic point of view.

Ossification has occurred in the trephine opening in the animal which had received the cerebrin. The meninges are thickened at the point of the incision. The sections show a microscopic picture somewhat similar to those described above. Hemorrhagic elements are present and the small blood vessels are prominent just as they were in the control and phospholipin specimens. A little more fibrosis has occurred in the area with a slight tendency to capsule formation. There is considerably more gliosis both of the fibril-forming and the cytoplastic type adjacent to the area of the lesion. The amount of phagocytosis is about the same as in the phospholipin experiment.

The gliosis was the most characteristic feature though it was not as marked as that seen in the cholesterol experiment (to be discussed later). It was quite decided and will probably justify further experiment.

Cholesterol. Cholesterol is a sterol or solid alcohol. It constitutes 17 per cent of dried corpus callosum (Koch) but it is also an important constituent of all cell protoplasm. Its empirical formula is $C_{27}H_{45}OH$. It is an unsaturated mono-alcohol and belongs to the general group of terpenes. It is associated with numerous physiologic and pathologic phenomena. Thus it is supposed to prevent hemolysis of the erythrocytes, to check lipolytic enzymes and in this way control the metabolism of fats, to be the mother substance of the bile salts and hence regulate fat absorption and to regulate the water metabolism of cells. It is one of the constituents of gall stones and is found in sclerosis of arteries. It is present in old degenerative processes such as old infarcts, exudates and degenerating tumors in the brain or in other parts of the body. Wells [6] thinks that its action in the cell must be purely a physical one since it is chemically inert. He also speaks of it as behaving like an inert foreign body in pathologic conditions and as being removed by giant cells and foamy endothelial cells. In the previous paper of this series it was shown that an experimental hypercholesterolemia had no effect on the brain tissues of rabbits. When it was introduced subdurally by lumbar puncture, however, there was a fibrous increase in the dura and in the cellular content of the arachnoid, with phagocytosis of the cholesterol and a slight gliosis in the subjacent spinal cord tissue.

In the dog brain there is no gross evidence of infection. The meninges are slightly thickened and adherent as in the other cases. The microscopic picture of the section is striking. Polymorpho-

nuclear cells are sprinkled around a few of the blood vessels and it will therefore be necessary to repeat this work to eliminate the possibility of infection, although these cells might have been called forth by the extract itself. A definite fibrous capsule has formed around the injected substance with ingrowths of fibrous tissue toward the center. The blood vessels around the area are thickened by an increase of their endothelial cells and fibroblasts. Numerous phagocytes are present but these are outside the fibrous capsule which walls off the cholesterol and seems to be associated with the hemorrhage and tissue injury. The gliosis both of the fiber-forming and cytoplastic type is marked, the latter being very conspicuous in places. Except for the gliosis this reaction corresponds closely with that of the spinal cord of the rabbits in which a fibrous increase in the dura and a cellular increase in the arachnoid were produced. The cholesterol was taken up by the phagocytes while in the brain of the dog it was walled off by the fibrous capsule. This difference in the glia reaction may be due to a difference in the substances injected as that used in rabbits was Merck's purified cholesterol while that used in the dog was extracted from a dog's brain.

Residue. The residue is the solid substance left after the brain has been extracted with hot alcohol and ether and may be considered to be the precipitated proteins. It is probably largely nucleo- or phosphoproteins and undoubtedly many other substances. Little is yet known of the nucleic acid of the brain. It yields on hydrolysis phosphoric acid, a purine base and a hexose (Mathews [1]).

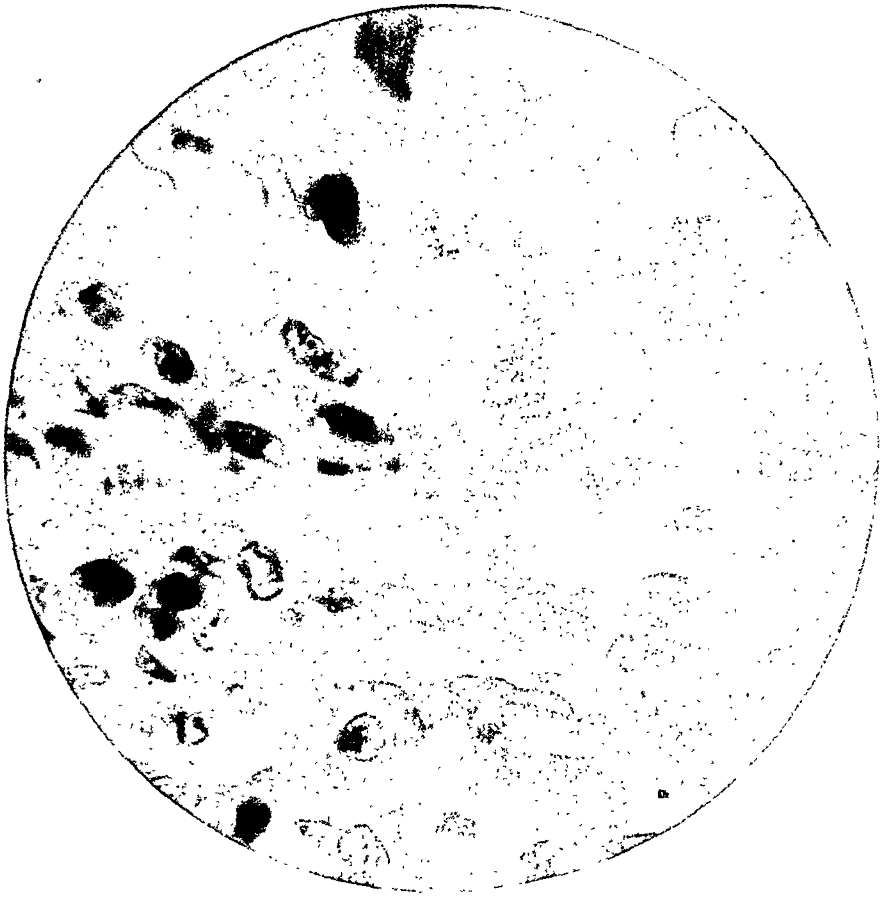
In the animal injected with this residue, the brain in gross looks like the others. Microscopically, a conspicuous fibrous capsule surrounds the injected mass like that in the cholesterol experiment. Outside of this capsule is seen some hemorrhage at one place and also a little tissue destruction. Strikingly few phagocytes occur and the gliosis of the brain tissue is less than in any of the specimens except the control. The striking part of the picture is the heavy fibrous capsule which differs from that in the cholesterol specimen in the number and form of the nuclei. These are numerous and very bizarre in their shape. In addition, a border of syncytial tissue with numerous nuclei and a frothy cytoplasm which seems to be invading the foreign substance with tongue-like projections has developed just inside the capsule. This tissue has been interpreted as a phagocytic syncytium. Such tissue formations are of very considerable

interest. The products of nuclein hydrolysis are said to stimulate cell growth (Wells,[6] and Calkins et al.[8]) and such an intense and bizarre nuclear reaction as seen here will certainly justify further studies with this substance and also further experiment to determine whether the active factor is the nucleic acid or some other ingredient.

Conclusions

One of the interesting results of these experiments is the way each picture overlaps the next. If we may consider each of the four extracts as a sort of fractional product with a predominating substance but containing lesser quantities of the others, we may explain the overlap.

The effect on the blood vessels is about the same in all of the series including the control. These vessels are more prominent in and about the area of the lesion and show an increase in the endothelial cells and the fibroblasts. Calcification was evident in the phospholipin and suggested in the phosphoprotein experiments. The phagocytic reaction varied directly as the amount of hemorrhage and tissue destruction and inversely as the amount of encapsulation. The fibrous reaction within the lesion and capsule formation were least in the phospholipin injection and a little greater in the cerebrin experiment, there being an indication of capsule formation. A definite capsule had developed in the cholesterol experiment with invasion of the mass by fibroblasts, but still numerous extracapsular phagocytes were to be found. In the residue experiment the capsule formation was complete. The rapid nuclear production was at its maximum as a reaction to the residue, though a slight suggestion of it occurred in the cholesterol experiment. On the other hand, the fibrous and invasive reactions were at the maximum with the cholesterol extract where bands of fibrous tissue invaded the mass. Gliosis was the least marked in the control and in the residue experiments. It was increased by cerebrin and was marked with phospholipin and perhaps even more so with cholesterol. The fact that purified cholesterol failed to produce any marked gliosis in the spinal cord of rabbits suggests that some substance common to the phospholipin and cholesterol extracts, which is not present in the purified material, serves as the glia stimulant.



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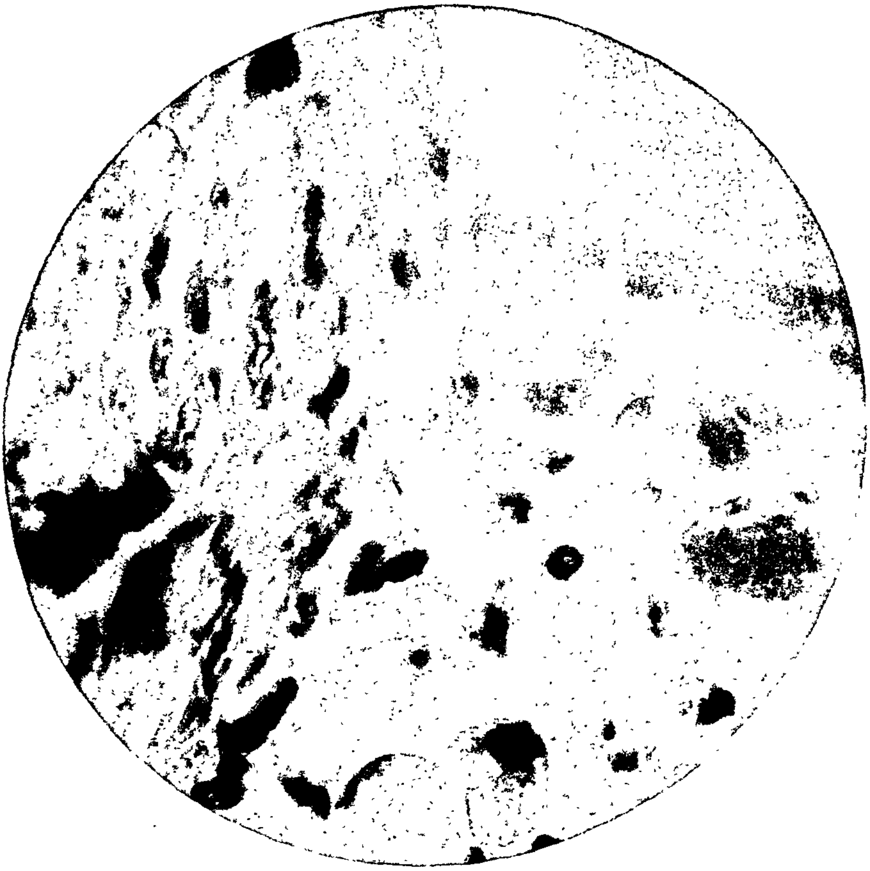

2

Bender

Experimental Gliosis

These experiments have to some degree separated the various constituents which usually form a single picture in a lesion in the brain. To separate these constituents more completely, it will be necessary to obtain either more nearly pure extracts or to fractionate these more completely. Studies in the various stages in the development of lesions resulting from the present series of extracts might also be of value. The water-soluble extracts were not used in these experiments and these may be important factors in the tissue reactions to lesions in the central nervous system. They include alkaloids, organic and inorganic acids, carbohydrates, amino acids and other unknown substances.

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DESCRIPTION OF PLATE CIV

FIG. 1. Oil immersion photomicrograph of the area of a dog's brain injected with the brain residue or nucleoproteins. At the lower edge of the picture is the brain tissue with the cellular and fibrous tissue which has encapsulated the injected mass and above it the phagocytic syncytial tissue which is invading the mass. The bizarre nuclear forms are well shown.

FIG. 2. Oil immersion photomicrograph of the injected brain residue or nucleoprotein (in the upper right-hand sector) surrounded by the syncytial phagocytic tissue (in the lower left-hand sector) and encapsulated by a fibrous tissue (on the left).

A MULTINUCLEATED LIVER CELL CARCINOMA

HENRY S. ROWEN AND F. B. MALLORY

(*From the Pathological Laboratory of the Boston City Hospital*)

The following example of liver cell carcinoma is reported on account of the unusual character of many of the cells composing it. No postmortem examination was permitted but the character of the tumor is perfectly evident from its histologic structure. The tissue examined was obtained by surgical operation at the St. Elizabeth's Hospital, Boston, and was brought to this laboratory for diagnosis.

Clinical History. O. O., a male, 57 years old, born in Sweden and a laborer by occupation, came to the hospital Feb. 27, 1925, complaining of pains in the right upper abdominal quadrant.

Family History. Negative.

Past History. Influenza in 1920. "Stomach trouble" since October, 1925. Failing appetite. Clay colored stools one week previous to entrance. Intermittent vomiting past month. No cardiac or respiratory symptoms. Frequent micturition.

Present Illness. Pains in right upper quadrant for past two months, extending the past two weeks to left upper quadrant and into both shoulder blades, accompanied on several occasions with nausea and vomiting.

Physical Examinations. Fairly well developed and nourished. Teeth in poor condition. Heart and lungs practically negative. There is a resistant mass felt in right upper quadrant, extending from the costal margin nearly to the umbilicus. This mass moves on deep inspiration and is somewhat tender on palpation.

Laboratory Examinations. Urinalysis negative. White blood count 8,600. Clotting time normal.

X-ray findings by Dr. L. B. Morrison were as follows:

"Gastro-intestinal meal given shows long tubular type stomach. First part of duodenum does not fill out, and pylorus acts as if pressed upon. At 6 hours head of column has reached ascending colon. There is a large amount of the meal in the jejunum and in the ileum which is dilated. At 24 hours head of column has reached the rectum, most of it is in the transverse colon. Pictures of gall

* Received for publication October 15, 1925.

bladder do not reveal any evidence of stone. There seems to be an increased density in gall bladder region. Liver seems large.

"Conclusions. The lack of filling in the pylorus and first part of duodenum I believe is due to some pressure here from something in the abdominal cavity. The ascending colon ought to be checked up by an enema. The dilatation of terminal ileum is due to adhesions in the lower right quadrant."

Operation March 9, 1925. Exploratory laparotomy under ether anesthesia. High right rectus incision; peritoneum opened whereupon there was a profuse gush of thin bloody fluid. Liver was examined and found to be considerably enlarged and entirely studded with relatively small and large cyst-like masses. When opened these masses were found filled with material of the color and consistency of wet cocoa. Small portion of liver excised. Gall bladder appeared normal. Further surgical interference considered useless. Peritoneum and fascia sutured with catgut and skin approximated with silkworm gut sutures. Sterile dressing applied. Recovery fair.

Three days following operation, wound discharged a large amount of bloody fluid which persisted up to the time of his death, March 21, 1925.

Microscopic Examination

Microscopic examination of sections from the liver shows an infiltrating tumor which is spreading everywhere through the smaller blood vessels and often causes marked distension of them. The new growth is composed of cells more or less closely resembling those of the liver but of a distinctly larger size. In places they are arranged in columns with sinusoids and very little stroma between them. Occasionally groups of them contain small and large fat vacuoles so that the picture strongly suggests that seen in a fatty infiltrated liver.

Under high power magnification it is sometimes difficult to distinguish tumor from liver parenchyma. With low magnification, however, the distinction is rendered easy by the larger size of the cells, the tendency to basophilic instead of acidophilic staining of the cytoplasm and the lack of lobular arrangement and bile duct formation.

In places the tumor cells surround bile capillaries of which many are distended with fluid and inspissated bile. The unusual feature

of the tumor, however, is the presence in many places of masses of multinucleated cells of various shapes. These cells are often of huge size and each contains several to dozens of nuclei which as a rule are separate but occasionally more or less fused together. In a few of the large cells are bile capillaries distended with inspissated bile.

Careful search reveals mitotic figures in small numbers. Some of them are single but others are multiple and clearly indicate the origin of the multinucleated cells which are true tumor giant cells.

A certain amount of sclerosis is present in the liver tissue between the masses of cancer cells but this may be due to the action of the tumor. Neither hemosiderin nor hyalin can be found in any of the liver cells.

Some of the tumor nodules are necrotic and undergoing solution. The cysts noted on fresh examination evidently arose in this manner from larger tumor nodules.

Discussion

Primary carcinomas of the liver are relatively rare. The records of this laboratory contain nine in 6506 consecutive postmortem examinations. Seven of them are of the liver cell type. Bile capillaries distended by cylinders of inspissated bile are present in some of them and one shows marked fatty infiltration of the tumor cells but none contains giant cells.

The following points of interest in connection with these seven tumors are worthy of note. Two gave rise to metastases in the regional lymph nodes and one was complicated by cancerous thrombosis of the inferior vena cava with metastases in the lungs and extension to the pleural cavities.

One patient was 32 years of age, the others ranged from 45 to 78. All were males but one. The point of greatest interest is the frequent association of this variety of tumor with cirrhosis. This close interrelation, usually put at over 80 per cent, has long been recognized. In six of these seven cases of liver cell cancer the cirrhosis is well marked. It is of the pigment type (hemochromatosis) in three and combined with hyalin (the so-called alcoholic type) in the other three. The seventh case is doubtful, not enough liver tissue free from tumor having been preserved to permit a positive statement. There is some sclerosis present but it may be due to the tumor just as in the new case reported in detail.

Cirrhosis of the liver, especially of the chronic progressive types (pigment and alcoholic), furnishes the cell changes favorable for the production of cancer. Continual necrosis of liver cells followed by regeneration results finally in the production of a cell free from control which proliferates indefinitely as a tumor.

The pigment type of cirrhosis, which requires probably the longest time for its production, some ten to twenty-five or more years, seems to be the variety most commonly associated with liver cell cancer. Besides the three marked cases cited here, tissues from two others have been sent to the laboratory and reports of similar examples occur in the literature.

Summary and Conclusions

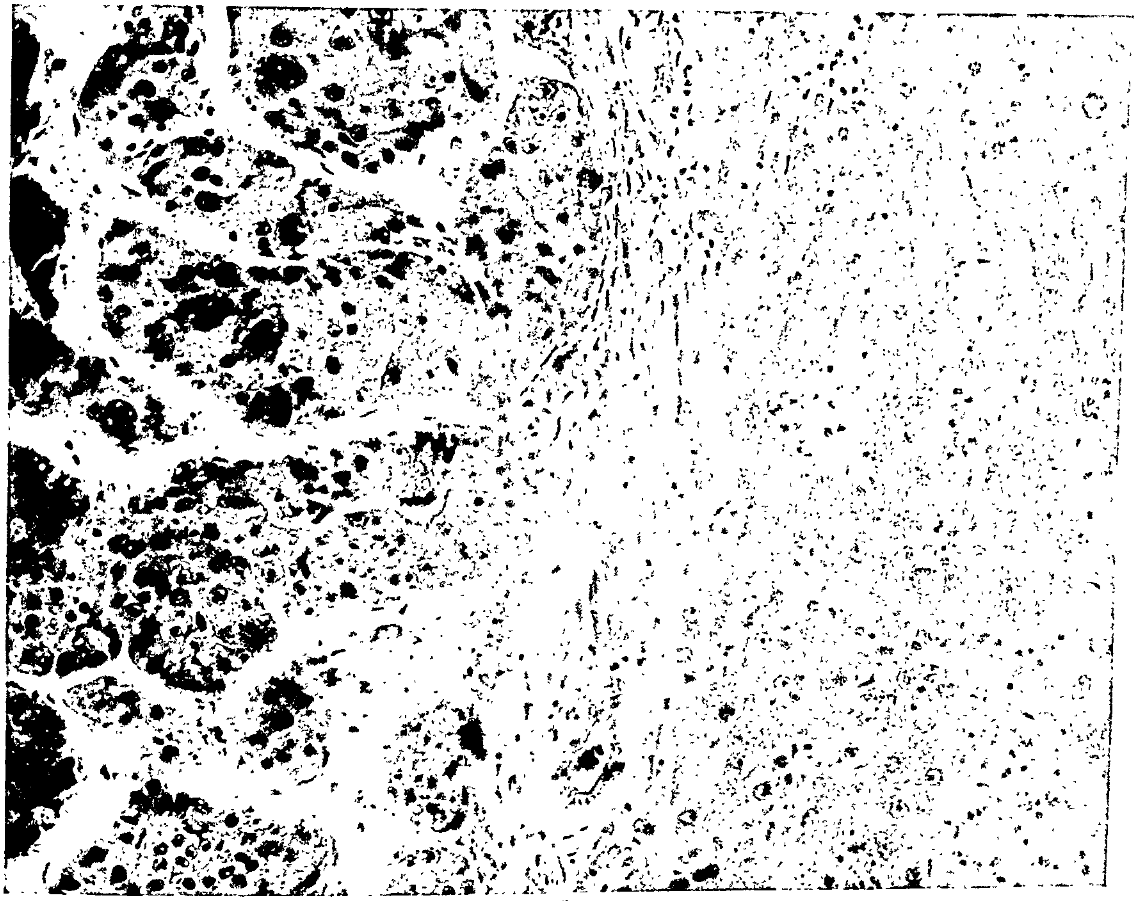
A report is given of a liver cell carcinoma containing many huge multinucleated tumor cells which have originated from multiple mitoses. In addition reference is made to seven other tumors of the same type, but without multinucleated cells, occurring in 6506 consecutive necropsies. Six of the eight cancers are associated with definite cirrhosis which is of the pigment type in three. In the other three, the cirrhosis is a combination of the pigment and alcoholic types.

Judging from these examples and others in the literature, pigment cirrhosis is the type which is most commonly complicated by liver cell carcinoma.

DESCRIPTION OF PLATES CV–CIX

FIG. 1. Liver on right side, tumor on left. x 200.
FIG. 2. Bile capillaries in tumor distended with fluid and inspissated bile. x 200.
FIG. 3. Two types of growth present, an area with cells containing single nuclei surrounded by multinucleated cells. The picture suggests a chorionepithelioma, but bile cylinders are present in bile capillaries. x 250.
FIG. 4. Multinucleated tumor cells: a multiple mitosis in lower center. x 250.
FIG. 5. Multinucleated tumor cells. x 250.
FIG. 6. A huge multinucleated tumor cell. x 300.
FIG. 7. Cylinders of inspissated bile in bile capillaries of tumor. x 500.
FIG. 8. A multinucleated tumor cell containing two bile cylinders. x 500.
FIG. 9. A large cell containing a nucleus with much chromatin material. x 1000.
FIGS. 10, 11 and 12. Multiple mitoses: FIG. 11 is a detail from Fig. 4. x 1000.

We are indebted to Miss Lillian M. Leavitt for assistance in making the photomicrographs.



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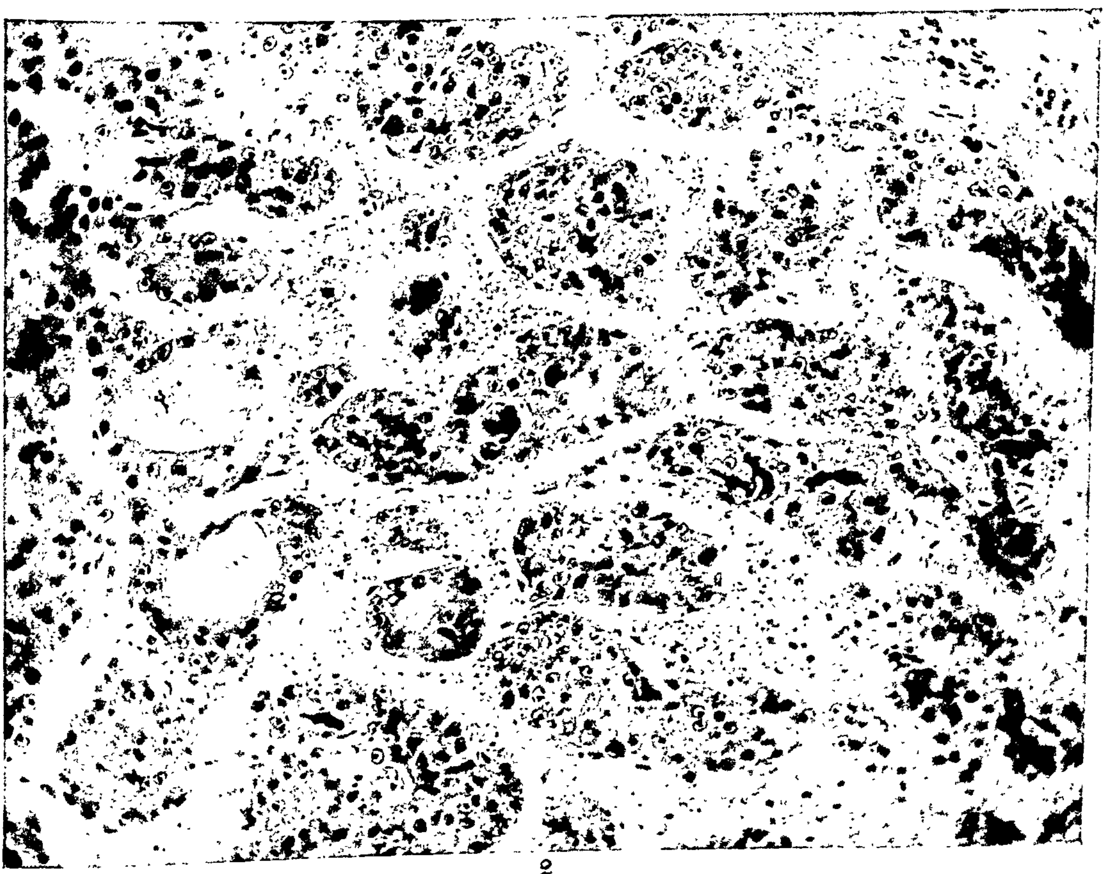

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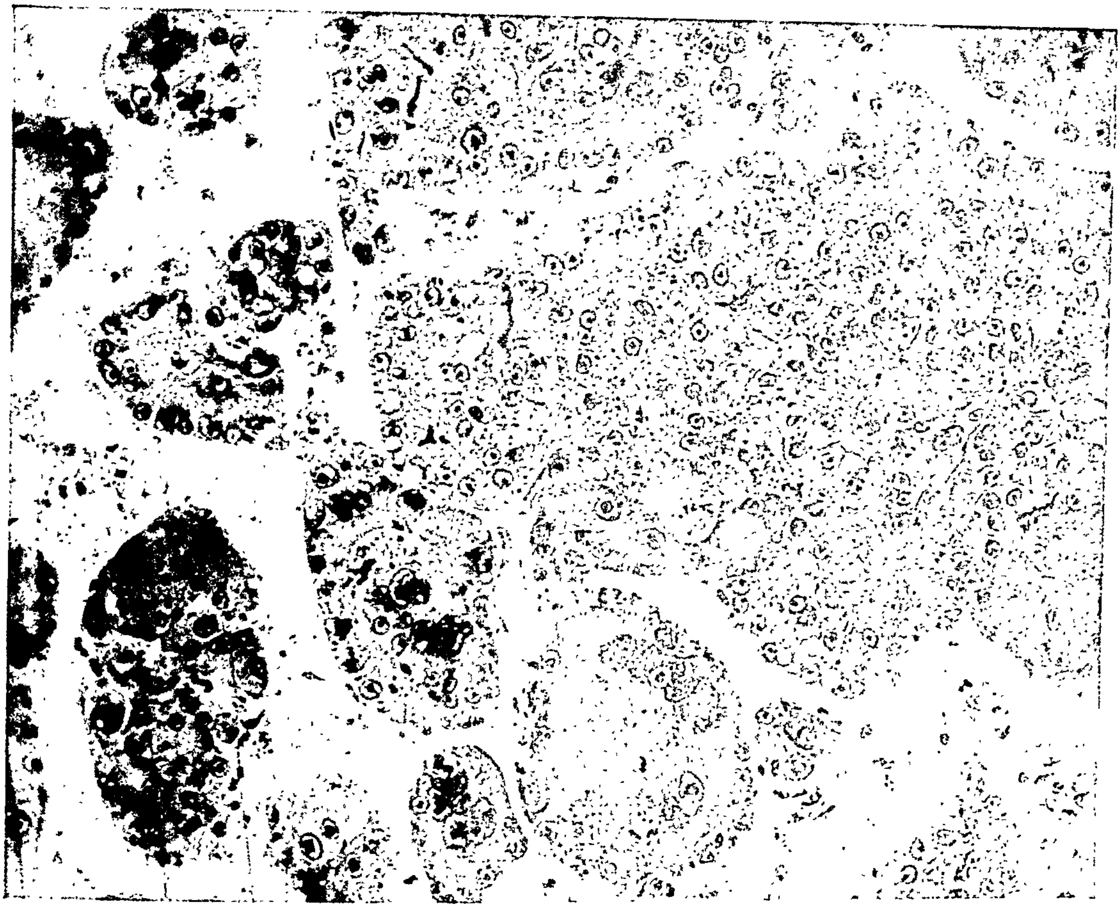
Rowen and Mallory

Multinucleated Liver Cell Carcinoma



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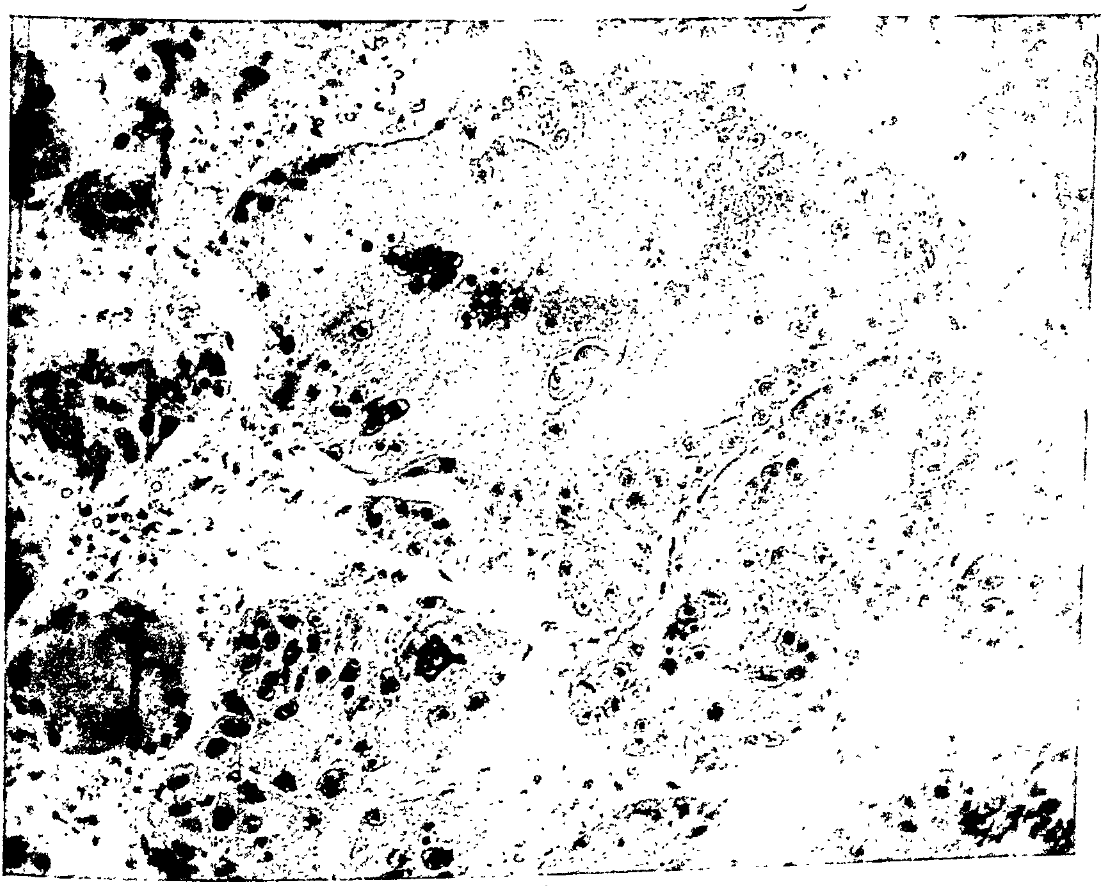

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Rowen and Mallory

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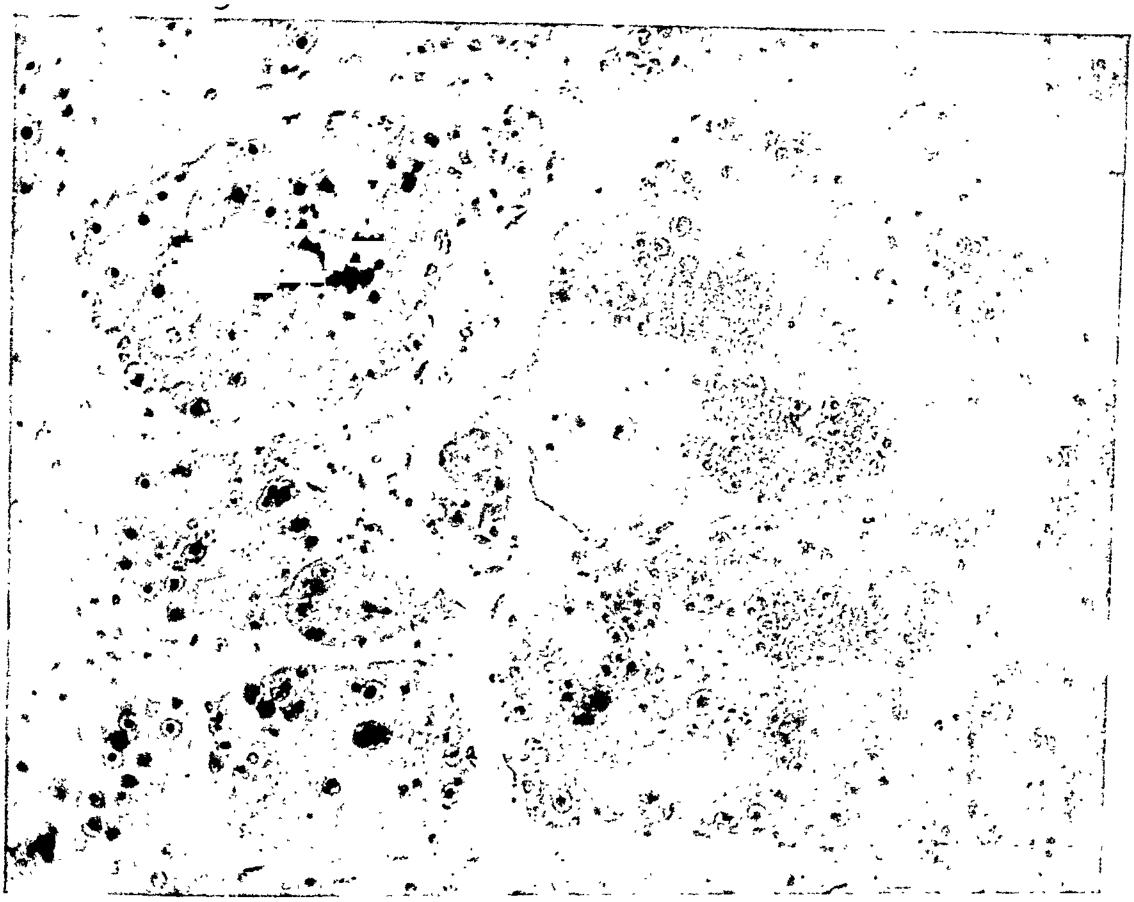

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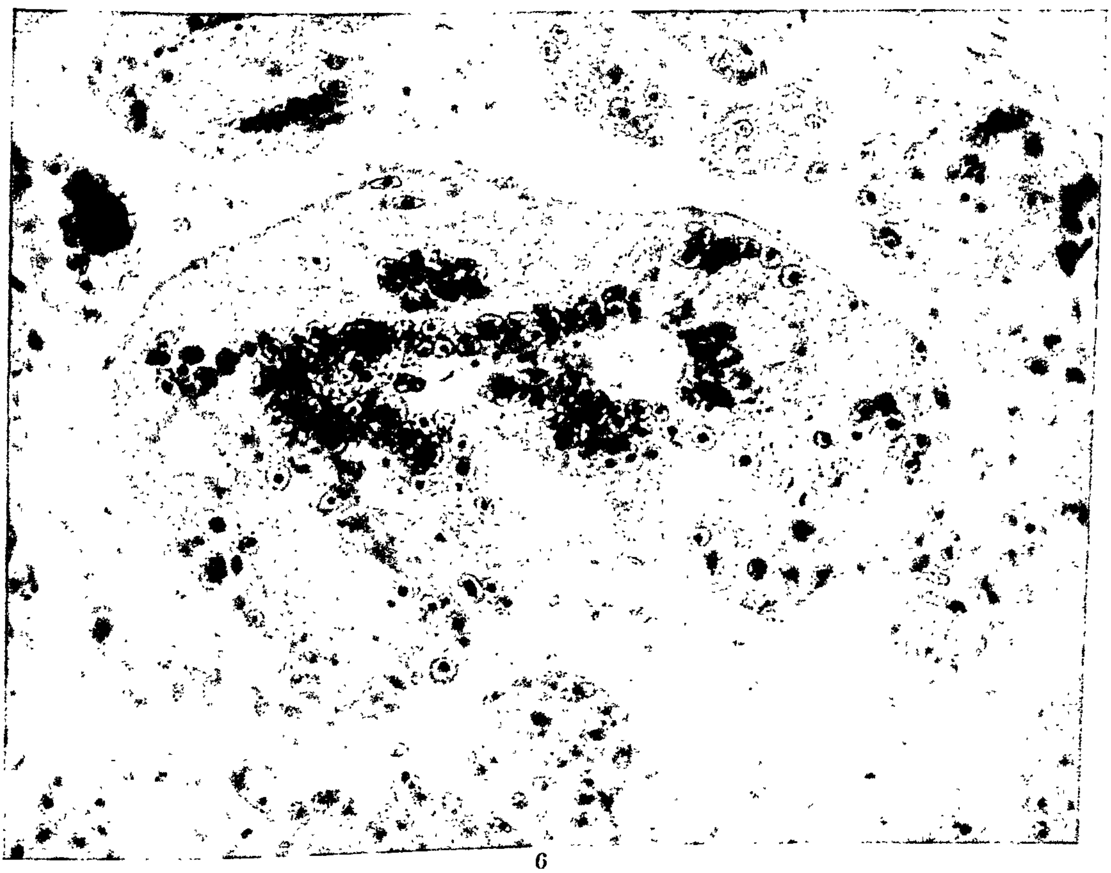

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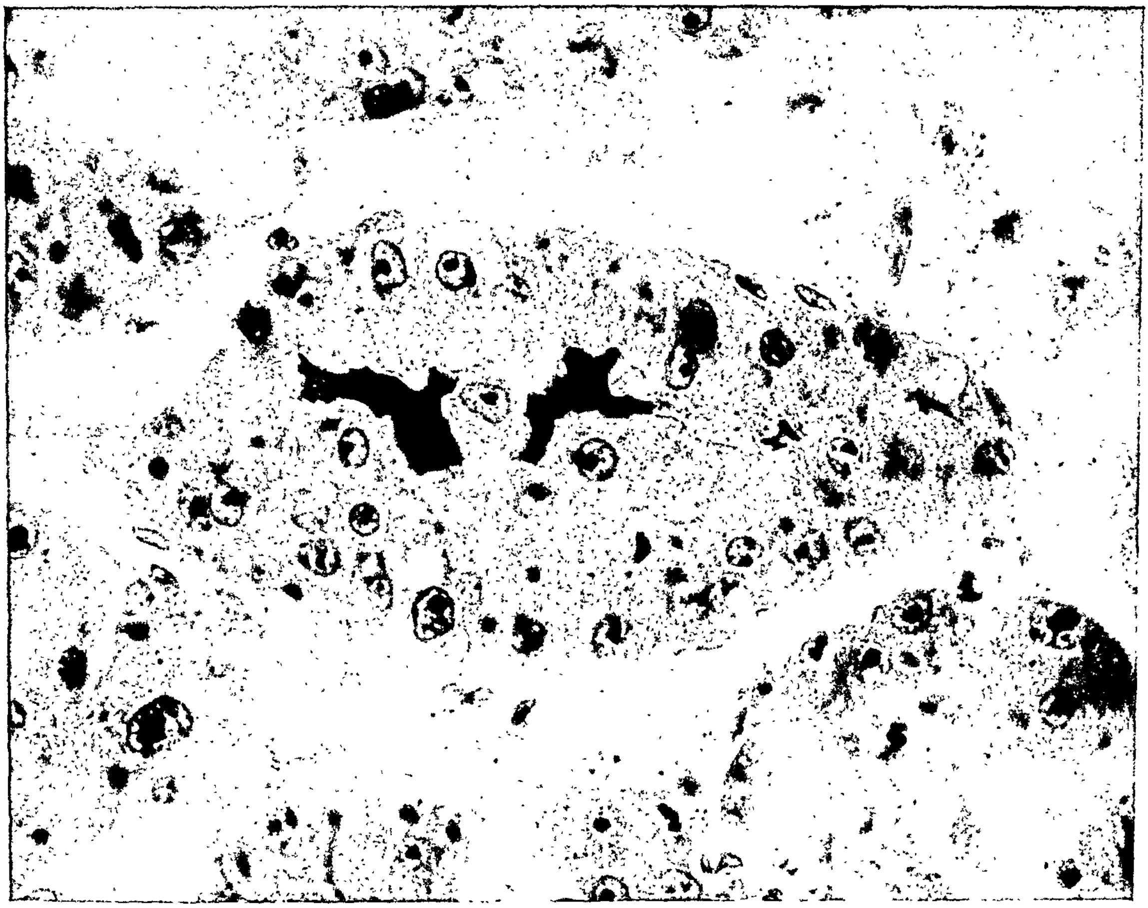
Rowen and Mallory

Multinucleated Liver Cell Carcinoma



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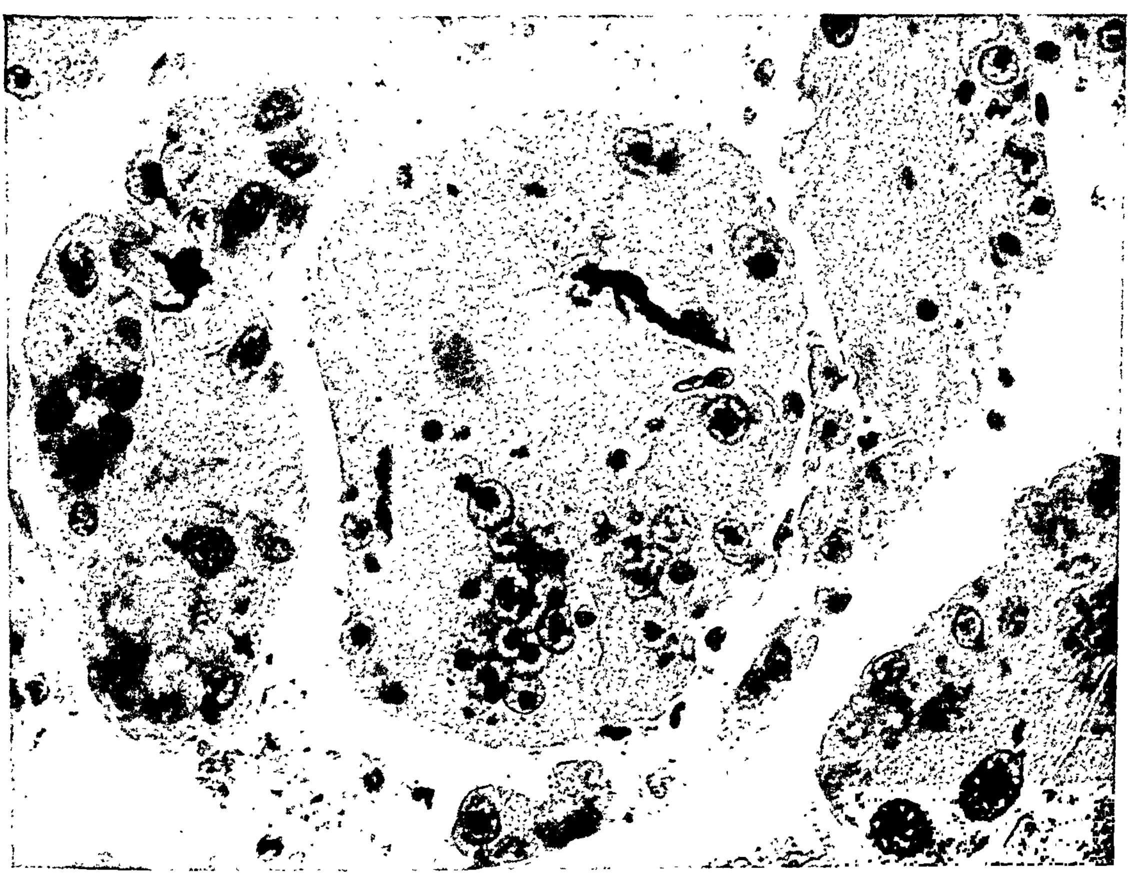

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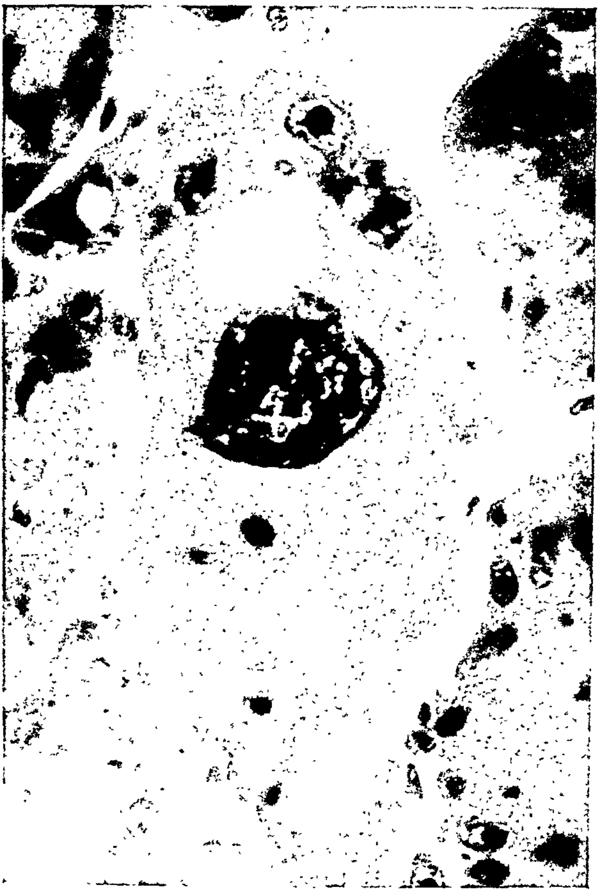
Rowen and Mallory Multinucleated Liver Cell Carcinoma



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Rowen and Mallory

Multinucleated Liver Cell Carcinoma

A CORRECTION*

N. C. Foot, M.D.

(*Cincinnati, Ohio*)

In an article on the occurrence of reticulum in tumors (Foot and Day, 1925), which appeared recently in this Journal, the following statement was made on p. 432: "As nothing appears to have been done in connection with the distribution and occurrence of reticulum in tumors, etc." As I was directly responsible for this statement and as I have received two communications that prove it to be erroneous, I wish to correct it at once.

It appears that White (1900) published an article on this subject from Mall's laboratory, setting forth the results of his investigation of tumor reticulum carried on by means of the Mall and the Spalteholtz digestion methods. His conclusions were very similar to ours, excepting that these methods did not enable him to distinguish clearly between collagen and reticulin.

Furthermore, there is an increasing amount of Italian literature on the subject of reticulum in tumors. Speciale (1924) has reviewed in "Tumori" the work done by some twelve investigators. Of these, one was Japanese and two Spanish, the rest being Italian. Among the articles he quotes, that of Niosi is strikingly similar to our own and our findings corroborate his in almost every particular. He used the Bielschowsky-Maresch impregnation which is essentially the same as the modification employed by us. He finds that reticulum is scarce in benign tumors and has no definite retiform arrangement; it is abundant in malignant growths but is found chiefly in the stroma of the carcinoma, unless that is invaded by wandering tumor cells in which case reticulum appears to be intra-alveolar. He draws attention to the close relationship between reticulum and the supporting tissue of new-growths and to its almost complete absence in the case of hypernephroma. Speciale's review of this article is so excellent that I have not read the original, but have nevertheless listed it at the end of this communication (Niosi, 1914).

Speciale reports his own investigation of a number of tumors in which he studied the reticulum by means of the Achúcarro modifi-

* Received for publication October 12, 1925.

cation of the de Rio-Hortega silver-tannate impregnation. His conclusions are as follows. Reticulum may be found in a great variety of tumors, be they benign or malignant, of connective tissue or epithelial origin; it is, therefore, of little value as a diagnostic landmark in distinguishing between them. It has a definite relationship to the vascular supply of tumors, more particularly to the capillaries and is, therefore, more probably a product of the capillary endothelium than of the tumor cells. He finds that the more mature is the tissue in which tumor reticulum occurs, the less there is of it; while collagen is more abundant in such mature tissue. He therefore stresses the interdependence of reticulin and collagen, believing that the latter is a product of the former, and he quotes Barbacci and Luna as having already emphasized this point.

Achúcarro (1911), in a general article on his silver-tannate method, describes two sets of preparations made from a sarcoma and a carcinoma and compares his results with those of Russakoff who used the Bielschowsky-Maresch method and whose work was quoted in our paper.

I am indebted to Drs. W. C. White and Percival Bailey for calling my attention to these omissions from our article. It is fortunate that our results so closely coincide with those of the authorities just cited. When employing any method of investigation that necessitates the use of a silver impregnation, it would be well to bear in mind that great strides have been made in this direction by the Spanish followers of Ramon y Cajal and by a number of Italian investigators. It is unfortunate that the Spanish and Italian literature is so generally inaccessible in the medical libraries of this country, a fact which largely accounts for our oversight in omitting these references from our article.

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INDEX OF SUBJECTS

INDEX OF SUBJECTS

A

Acoustic nerve. — The fibroma of the . . . (*Jacobson*) - - - - - - - 259
Acromegaly. — Concerning the microscopic structure of the hypophysis cerebri in . . . (*Bailey and Davidoff*) - - - - - - - - - - - - 185
Adenocarcinoma. — An . . . resembling the thyroid gland (*Feldman*) - 281
Alcoholic. — Progressive . . . cirrhosis (*Hall and Ophüls*) - - - - - - 477
Anaphylaxis. — Rapid alterations in the blood sugar level of rabbits in . . . and following injections of bacterial proteins (*Zeckwer*) - - - 531
Anemia. — A contribution to the knowledge of splenic . . . (*Stillman*) - 541
Anemia. — Intestinal obstruction and pernicious . . . Report of a case (*Haden*) - 542
Anemia. — Phagocytosis of erythrocytes in the bone marrow, with special reference to pernicious . . . (*Peabody and Brown*) - - - - - - - 169
Aorta. — The effect of certain metabolic changes upon the . . . of rabbits and guinea-pigs (*Saphir*) - - - - - - - - - - - - - - - - - 403
Appendicitis. — The etiology of acute . . . (*Warren*) - - - - - - - - 241
Appendix. — Bilharzia in the . . . (*Plaut*) - - - - - - - - - - - - 532
Appendix. — Mucocele of the . . . with globoid body formation (*Milliken and Poindexter*) - - - - - - - - - - - - - - - - - - - 397
Arachnid. — . . . infection in monkeys (*Helwig*) - - - - - - - - - - 389
Atrophy. — The state of the cardiac muscle in hypertrophy and . . . (*Karsner, Saphir and Todd*) - - - - - - - - - - - - - - - - 351

B

B. paratyphosus B. — The pathological effects of soluble toxic substances obtained from . . . (*Menten*) - - - - - - - - - - - - - - - 539
Bilharzia. — . . . in the appendix (*Plaut*) - - - - - - - - - - - - - 532
Biliary. — The pathogenesis of . . . calculi (*Mentzer*) - - - - - - - 538
Blood. — The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated . . . (*Lewis*) - - - - - - - - - 91
Blood. — Studies of the . . . in an epidemic of smallpox (*Ikeda*) - - - 542
Blood. — Studies on . . . fibrin (*Schultz, Nicholes and Schaefer*) - - - 101
Blood sugar. — Rapid alterations in the . . . level of rabbits in anaphylaxis and following injections of bacterial proteins (*Zeckwer*) - - - 531
Body. — Riedel's struma associated with remnants of the post-branchial . . . (*Meeker*) - 57
Bone marrow. — Heterotopia of the . . . without apparent cause (*Saleeby*) - 69

Bone marrow. — Phagocytosis of erythrocytes in the . . . with special reference to pernicious anemia (*Peabody and Broun*) - - - - - - 169

Brain. — Ependymomas of the lateral and fourth ventricles of the . . . (*Hirsch and Elliott*) - - - - - - - - - - - - - - - - - 627

Brain. — Experimental production of gliosis. II. Reaction of . . . tissue to the lipoid fractions and to the residue of . . . extracts (*Bender*) - 667

C

Calculi. — The pathogenesis of biliary . . . (*Mentzer*) - - - - - - - 538

Cancer. — Mechanical irritation as etiologic factor of . . . (*Alter*) - - 511

Carcinoma. — A multinucleated liver cell . . . (*Rowen and Mallory*) - 677

Carcinoma. — Unusual modes of dissemination of ovarian . . . (*Levin and Barnet*) - - - - - - - - - - - - - - - - - - - 534

Cardiac muscle. — The state of the . . . in hypertrophy and atrophy (*Karsner, Saphir and Todd*) - - - - - - - - - - - - - 351

Cardiac puncture. — Hematocele of the wall of the atrium following . . . (*Schultz*) - 225

Cardio-vascular. — On the incidence of acute and subacute infective inflammatory processes in . . . defects and on malformed semilunar cusps. With a statistical review of the literature and reports of six cases, in three of which was a fungating mycotic endarteritis of the pulmonary trunk (*Abbott*) - - - - - - - - - - - - - - 543

Cholesterol. — Experimental production of gliosis. I. Effects on the nervous system of the rabbit of intravenous and intraspinal injections of . . . emulsion (*Bender*) - - - - - - - - - - - - - - 657

Cholesterosis. — . . . of the gall bladder (*Mentzer*) - - - - - - - 383

Cirrhosis. — Progressive alcoholic . . . (*Hall and Ophüls*) - - - - - 477

Congestion. — The histological alterations of the pancreas in chronic passive . . . (*Von Glahn and Chobot*) - - - - - - - - - - 373

Conjunctival. — Epithelial cells in . . . infections (*McKee*) - - - - 536

Copper. — The relation of chronic poisoning with . . . to hemochromatosis (*Mallory*) - - - - - - - - - - - - - - - - - - 117

Correction. — A . . . (*Foot*) - - - - - - - - - - - - - - - - 681

D

Diabetes. — Evidence of regeneration of pancreas in an insulin treated case of . . . (*Boyd and Robinson*) - - - - - - - - - - - 135

Diabetes. — The pathology of . . . with special reference to pancreatic regeneration (*Warren and Root*) - - - - - - - - - - - - 415

Dick test. — Further results with the . . . and active immunization with scarlatinal toxin (*Zingher*) - - - - - - - - - - - - - 530

Diphtheria. — . . . immunity. The effect of repeated injections of avirulent . . . bacilli, B. Hofmanni and B. Xerosis in guinea-pigs (*Rosenau and Bailey*) - - - - - - - - - - - - - - - - - 529

E

Edema. — Gross and histologic changes in the . . . of paraphenylenediamine (*Tainter and Hall*) - - - - - - - - - - - - - - - - 503

Encephalitis. — Certain factors determining the incidence and severity of herpetic . . . in rabbits (*Goodpasture*) - - - - - - - - - - - - 47

Encephalitis. — Localization of streptococci from cases of epidemic nausea and vomiting and of epidemic neuro-myelo- . . . (*Rosenow*) - 539

Encephalitis. — The pathways of infection of the central nervous system in herpetic . . . of rabbits contracted by contact; with a comparative comment on medullary lesions in a case of human poliomyelitis (*Goodpasture*) - 29

Endarteritis. — On the incidence of acute and subacute infective inflammatory processes in cardio-vascular defects and on malformed semilunar cusps. With a statistical review of the literature and reports of six cases, in three of which was a fungating mycotic . . . of the pulmonary trunk (*Abbott*) - - - - - - - - - - - - - - - - - 543

Endocarditis. — Rheumatic . . . as related to subacute bacterial . . . (*Clawson*) - 542

Endometriosis. — Experimental . . . (*Jacobson*) - - - - - - - - - 537

Endothelial Reactions. — Studies on . . . IX. The formation of reticulum in the lesions of experimental tuberculosis in rabbits (*Foot*) - - 341

Ependymomas. — . . . of the lateral and fourth ventricles of the brain (*Hirsch and Elliott*) - - - - - - - - - - - - - - - - - - - 627

Epidemic nausea. — Localization of streptococci from cases of . . . and vomiting and of epidemic neuro-myelo-encephalitis (*Rosenow*) - - 539

Epithelial. — . . . cells in conjunctival infections (*McKee*) - - - - - - 536

Epithelial. — The . . . tissues in experimental xerophthalmia (*Wolbach and Howe*) - 536

Epithelioid cells. — The formation of macrophages, . . . and giant cells from leucocytes in incubated blood (*Lewis*) - - - - - - - - - 91

Erythrocytes. — Phagocytosis of . . . in the bone marrow, with special reference to pernicious anemia (*Peabody and Broun*) - - - - - - - 169

Exudates. — The origin of the mononuclear phagocytes of peritoneal . . . (*McJunkin*) - - - - - - - - - - - - - - - - - - - 305

F

Fibrin. — Studies on blood . . . (*Schultz, Nicholes and Schaefer*) - - - 101

Fibroma. — The . . . of the acoustic nerve (*Jacobson*) - - - - - - - 259

Filterable viruses. — Studies on . . . I (*Parker and Nye*) - - - - - - 325

Filterable viruses. — Studies on . . . II (*Parker and Nye*) - - - - - 337

Flocculation test. — The Vernes . . . as a guide to treatment of syphilis (*Baylis, Sheplar and MacNeal*) - - - - - - - - - - - - - - 529

G

Gall bladder. — Cholesterosis of the . . . (*Mentzer*) - - - - - - - - - 383

Germanium dioxide. — The action of . . . on the rabbit (*Bunting, Bailey and Davidson*) - 541

Giant cells. — The formation of macrophages, epithelioid cells and . . . from leucocytes in incubated blood (*Lewis*) - - - - - - - - - 91

Gliomas. — Microglia and the process of phagocytosis in . . . (*Penfield*) 77

Gliosis. — Experimental production of . . . I. Effects on the nervous system of the rabbit of intravenous and intraspinal injections of cholesterol emulsion (*Bender*) - - - - - - - - - - - - - - - - 657

Gliosis. — Experimental production of . . . II. Reaction of brain tissue to the lipoid fractions and to the residue of brain extracts (*Bender*) - 667

Glomerulonephritis. — Experimental . . . (*Bell, Clawson and Hartzell*) - 247

Glomerulonephritis. — Experimental . . . in monkeys (*Bell and Clawson*) 540

Glomerulonephritis. — Experimental intracapillary . . . (*Mallory and Parker*) - 541

Goitre. — The origin of adenomatous . . . (*Kline*) - - - - - - - - 235

H

Heart. — Congenital . . . disease with partial situs inversus, absence of inferior vena cava, and other anomalies (*Miller*) - - - - - - - - 467

Heart. — Spontaneous rupture of . . . based on thirteen unpublished cases and six hundred and twenty from the literature (*Krumbhaar and Crowell*) - 544

Hematocele. — . . . of the wall of the atrium following cardiac puncture (*Schultz*) - 225

Hemochromatosis. — The relation of chronic poisoning with copper to . . . (*Mallory*) - 117

Herpes. — The axis-cylinders of peripheral nerves as portals of entry to the central nervous system for the virus of . . . simplex in experimentally infected rabbits (*Goodpasture*) - - - - - - - - - - - 11

Herpetic. — Certain factors determining the incidence and severity of . . . encephalitis in rabbits (*Goodpasture*) - - - - - - - - - - 47

Herpetic. — Intranuclear inclusions in experimental . . . lesions of rabbits (*Goodpasture*) - - - - - - - - - - - - - - - - - - - 1

Herpetic. — The pathways of infection of the central nervous system in . . . encephalitis of rabbits contracted by contact; with a comparative comment on medullary lesions in a case of human poliomyelitis (*Goodpasture*) - 29

Heterotopia. — . . . of the bone marrow without apparent cause (*Saleeby*) 69

Hydatid cyst. — The . . . (*Cameron and Fitzpatrick*) - - - - - - - - 227

Hypersensitiveness. — Observations on focal tuberculin . . . in an infected organ previous to a general sensitization (*Stewart*) - - - - 495

Hypertrophy. — Compensatory . . . of the thymus gland (*Marmorston-Gottesman and Jaffe*) - - - - - - - - - - - - - - - - - - 536

Hypertrophy. — The state of the cardiac muscle in . . . and atrophy (*Karsner, Saphir and Todd*) - - - - - - - - - - - - - - - - 351

Hypophysis. — The pathology of the . . . I. The presence of abnormal cells in the posterior lobe (*Simonds and Brandes*) - - - - - - - 209

Hypophysis. — The pathology of the . . . II. Lymphocytic infiltration (*Simonds and Brandes*) - - - - - - - - - - - - - - - - - - - 273

Hypophysis cerebri. — Concerning the microscopic structure of the . . . in acromegaly (*Bailey and Davidoff*) - - - - - - - - - - - - - 185

I

Immunity. — Diphtheria . . . The effect of repeated injections of avirulent diphtheria bacilli, B. Hofmanni and B. Xerosis in guinea-pigs (*Rosenau and Bailey*) - - - - - - - - - - - - - - - - - - - 529

Immunization. — Further results with the Dick test and active . . . with scarlatinal toxin (*Zingher*) - - - - - - - - - - - - - - - 530

Inclusions. — Intranuclear . . . in experimental herpetic lesions of rabbits (*Goodpasture*) - 1

Inferior vena cava. — Congenital heart disease with partial situs inversus, absence of . . . , and other anomalies (*Miller*) - - - - - - - - 467

Insulin. — Evidence of regeneration of pancreas in an . . . treated case of diabetes (*Boyd and Robinson*) - - - - - - - - - - - - - - - 135

Intestinal. — . . . obstruction and pernicious anemia. Report of a case (*Haden*) - 542

Intestine. — Neuroblastoma of the . . . (*Ritter*) - - - - - - - - - - 519

Intranuclear inclusions. — . . . in visceral disease (*Von Glahn and Pappenheimer*) - 445

K

Kala-azar. — The histopathology of . . . in the hamster, monkey and man (*Meleney*) - 147

L

Leucocytes. — The formation of macrophages, epithelioid cells and giant cells from . . . in incubated blood (*Lewis*) - - - - - - - - 91

Lipoid-histiocytosis. — Splenomegaly (type Gaucher) and . . . (type Niemann). (*Bloom*) - 595

Liver. — A multinucleated . . . cell carcinoma (*Rowen and Mallory*) - - 677

M

Macrophages. — The formation of . . ., epithelioid cells and giant cells from leucocytes in incubated blood (*Lewis*) - - - - - - - - - 91

Mechanical irritation. — . . . as etiologic factor of cancer (*Alter*) - - - 511

Meninges. — Diffuse tumors of the . . . (*Boyd*) - - - - - - - - - - 583

Metabolic. — The effect of certain . . . changes upon the aorta of rabbits and guinea-pigs (*Saphir*) - - - - - - - - - - - - - - - - - - 403

Methylene blue. — Chemical studies on polychrome . . . (*MacNeal and Killian*) - 537

Microglia. — . . . and the process of phagocytosis in gliomas (*Penfield*) - 77

Mitochondria. — Studies on . . . (*Seecof*) - - - - - - - - - - - - 295

Monkeys. — Experimental glomerulonephritis in . . . (*Bell and Clawson*) 540

Mucocele. — . . . of the appendix with globoid body formation (*Milliken and Poindexter*) - 397

Mycotic. — On the incidence of acute and subacute infective inflammatory processes in cardio-vascular defects and on malformed semilunar cusps. With a statistical review of the literature and reports of six cases, in three of which was a fungating . . . endarteritis of the pulmonary trunk (*Abbott*) - - - - - - - - - - - - - - - - - 543

N

Negri bodies. — A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of . . . (*Goodpasture*) - 547

Neoplasms. — Renal . . . in young children (*Wollstein*) - - - - - - 534

Nerves. — The axis-cylinders of peripheral . . . as portals of entry to the central nervous system for the virus of herpes simplex in experimentally infected rabbits (*Goodpasture*) - - - - - - - - - - - - 11

Nervous system. — Experimental production of gliosis. I. Effects on the . . . of the rabbit of intravenous and intraspinal injections of cholesterol emulsion (*Bender*) - - - - - - - - - - - - - - - - 657

Neuroblastoma. — . . . of the intestine (*Ritter*) - - - - - - - - - - 519

Neuroma. — Plexiform . . . of the pharyngeal mucous membrane (*Meeker*) - 533

O

Obstruction. — Intestinal . . . and pernicious anemia. Report of a case (*Haden*) - 542

Oil. — Studies on pneumonia following naso-pharyngeal injections of . . . (*Laughlen*) - 407

Ovarian. — Unusual modes of dissemination of . . . carcinoma (*Levin and Barnet*) - 534

P

Pancreas. — Evidence of regeneration of . . . in an insulin treated case of diabetes (*Boyd and Robinson*) - - - - - - - - - - - - - 135

Pancreas. — The histological alterations of the . . . in chronic passive congestion (*Von Glahn and Chobot*) - - - - - - - - - - - - 373

Pancreatic. — The pathology of diabetes, with special reference to . . . regeneration (*Warren and Root*) - - - - - - - - - - - - - - 415

Paraphenylenediamine. — Gross and histologic changes in the edema of . . . (*Tainter and Hall*) - - - - - - - - - - - - - - - - - - 503

Parathyroidectomy. — The hypertrophy of the submaxillary gland in the albino rat following . . . (*Appleton*) - - - - - - - - - - - - - 217

Pathology. — The . . . of the hypophysis. I. The presence of abnormal cells in the posterior lobe (*Simonds and Brandes*) - - - - - - - - 209

Pathology. — The . . . of the hypophysis. II. Lymphocytic infiltration (*Simonds and Brandes*) - - - - - - - - - - - - - - - - - - 273

Pernicious. — Intestinal obstruction and . . . anemia. Report of a case (*Haden*) - 542

Pernicious. — Phagocytosis of erythrocytes in the bone marrow, with special reference to . . . anemia (*Peabody and Brown*) - - - - - - 169

Phagocytes. — The origin of the mononuclear . . . of peritoneal exudates (*McJunkin*) - 305

Phagocytosis. — Microglia and the process of . . . in gliomas (*Penfield*) - 77

Phagocytosis. — . . . of erythrocytes in the bone marrow, with special reference to pernicious anemia (*Peabody and Brown*) - - - - - - - 169

Pneumonia. — Studies on . . . following naso-pharyngeal injections of oil (*Laughlen*) - 407

Poisoning. — The relation of chronic . . . with copper to hemochromatosis (*Mallory*) - 117

Poliomyelitis. — The pathways of infection of the central nervous system in herpetic encephalitis of rabbits contracted by contact; with a comparative comment on medullary lesions in a case of human . . . (*Goodpasture*) - 29

Polychrome. — Chemical studies on . . . methylene blue (*MacNeal and Killian*) - 537

Post-branchial. — Riedel's struma associated with remnants of the . . . body (*Meeker*) - 57

R

Rabbit. — Spontaneous central nervous system lesions in the laboratory . . . (*Bender*) - 653

Rabbits. — The axis-cylinders of peripheral nerves as portals of entry to the central nervous system for the virus of herpes simplex in experimentally infected . . . (*Goodpasture*) - - - - - - - - - - - - 11

Rabbits. — Certain factors determining the incidence and severity of herpetic encephalitis in . . . (*Goodpasture*) - - - - - - - - - - 47

Rabbits. — Intranuclear inclusions in experimental herpetic lesions of . . . (*Goodpasture*) - 1

Rabbits. — The pathways of infection of the central nervous system in herpetic encephalitis of . . . contracted by contact; with a comparative comment on medullary lesions in a case of human poliomyelitis (*Goodpasture*) - - - - - - - - - - - - - - - - - - 29

Rabies. — A study of . . ., with reference to a neural transmission of the virus in rabbits, and the structure and significance of Negri bodies (*Goodpasture*) - 547

Rat. — The hypertrophy of the submaxillary gland in the albino . . . following parathyroidectomy (*Appleton*) - - - - - - - - - - 217

Regeneration. — Evidence of . . . of pancreas in an insulin treated case of diabetes (*Boyd and Robinson*) - - - - - - - - - - - - 135

Regeneration. — The pathology of diabetes, with special reference to pancreatic . . . (*Warren and Root*) - - - - - - - - - - - - - 415

Renal. — . . . neoplasms in young children (*Wollstein*) - - - - - - - 534

Reticulum. — The occurrence of . . . in tumors (*Foot and Day*) - - - 431

Reticulum. — Studies on endothelial reactions. IX. The formation of . . . in the lesions of experimental tuberculosis in rabbits (*Foot*) - - 341

Rheumatic. — . . . endocarditis as related to subacute bacterial endocarditis (*Clawson*) - - - - - - - - - - - - - - - - - - - 542

Riedel's. — . . . struma associated with remnants of the post-branchial body (*Meeker*) - 57

Roentgen irradiation. — Tissue changes after experimental deep . . . (*Kolodny*) - 285

Rupture. — Spontaneous . . . of heart based on thirteen unpublished cases and six hundred and twenty from the literature (*Krumbhaar and Crowell*) - 544

S

Sarcoma. — Congenital . . . of the leg (bone . . .?). Report of a case with eight years' cure (*Kolodny*) - - - - - - - - - - - - - 533

Scarlatinal toxin. — Further results with the Dick test and active immunization with . . . (*Zingher*) - - - - - - - - - - - - - - 530

Scorbutic. — The effect of the . . . state upon the production and maintenance of intercellular substances (*Wolbach and Howe*) - - - - 535

Situs inversus. — Congenital heart disease with partial . . . absence of inferior vena cava, and other anomalies (*Miller*) - - - - - - - 467

Smallpox. — Studies of the blood in an epidemic of . . . (*Ikeda*) - - - 542

Splenic. — A contribution to the knowledge of . . . anemia (*Stillman*) - 541

Splenomegaly. — . . . (type Gaucher) and lipoid-histiocytosis (type Niemann). (*Bloom*) - - - - - - - - - - - - - - - - - - - 595

Streptococci. — Localization of . . . from cases of epidemic nausea and vomiting and of epidemic neuro-myelo-encephalitis (*Rosenow*) - - 539

Struma. — Riedel's . . . associated with remnants of the post-branchial body (*Meeker*) - 57

Submaxillary gland. — The hypertrophy of the . . . in the albino rat following parathyroidectomy (*Appleton*) - - - - - - - - - - - 217

Syphilis. — The Vernes flocculation test as a guide to treatment of . . . (*Baylis, Sheplar and MacNeal*) - - - - - - - - - - - - - 529

T

Thymus gland. — Compensatory hypertrophy of the . . . (*Marmorston-Gottesman and Jaffe*) - - - - - - - - - - - - - - - - - - 536
Thyroid gland. — An adenocarcinoma resembling the . . . (*Feldman*) - 281
Tuberculin. — Observations on focal . . . hypersensitiveness in an infected organ previous to a general sensitization (*Stewart*) - - - - 495
Tuberculosis. — Extracts of normal tissues in experimental . . . (*Austin*) 539
Tuberculosis. — Studies on endothelial reactions. IX. The formatión of reticulum in the lesions of experimental . . . in rabbits (*Foot*) - - 341
Tumors. — Diffuse . . . of the meninges (*Boyd*) - - - - - - - - - 583
Tumors. — The occurrence of reticulum in . . . (*Foot and Day*) - - - 431

V

Vaccine virus. — Researches on . . . *in vitro* with special references to its affinity for nerve tissue (*Hirano*) - - - - - - - - - - - - - 635
Vernes. — The . . . flocculation test as a guide to treatment of syphilis (*Baylis, Sheplar and MacNeal*) - - - - - - - - - - - - - 529
Vomiting. — Localization of streptococci from cases of epidemic nausea and . . . and of epidemic neuro-myelo-encephalitis (*Rosenow*) - - 539

X

Xerophthalmia. — The epithelial tissues in experimental . . . (*Wolbach and Howe*) - 536

INDEX OF AUTHORS

INDEX OF AUTHORS

A

Abbott, Maude E. On the incidence of acute and subacute infective inflammatory processes in cardio-vascular defects and on malformed semilunar cusps. With a statistical review of the literature and reports of six cases, in three of which was a fungating mycotic endarteritis of the pulmonary trunk 543

Alter, Nicholas M. Mechanical irritation as etiologic factor of cancer . . 511

Appleton, Joseph L. The hypertrophy of the submaxillary gland in the albino rat following parathyroidectomy 217

Austin, Richard S. Extracts of normal tissues in experimental tuberculosis 539

B

Bailey, G. H. See Rosenau and Bailey. 529

——. See Bunting, Bailey and Davidson 541

Bailey, Percival, and Davidoff, Leo M. Concerning the microscopic structure of the hypophysis cerebri in acromegaly 185

Barnet, Joseph. See Levin and Barnet 534

Baylis, A. B., Sheplar, A. E., and MacNeal, W. J. The Vernes flocculation test as a guide to treatment of syphilis 529

Bell, E. T., and Clawson, B. J. Experimental glomerulonephritis in monkeys 540

——, ——, and Hartzell, T. B. Experimental glomerulonephritis 247

Bender, Lauretta. Spontaneous central nervous system lesions in the laboratory rabbit 653

——. Experimental production of gliosis. I. Effects on the nervous system of the rabbit of intravenous and intraspinal injections of cholesterol emulsion 657

——. Experimental production of gliosis. II. Reaction of brain tissue to the lipoid fractions and to the residue of brain extracts. 667

Bloom, William. Splenomegaly (type Gaucher) and lipoid-histiocytosis (type Niemann) 595

Boyd, Gladys L., and Robinson, W. L. Evidence of regeneration of pancreas in an insulin treated case of diabetes 135

Boyd, William. Diffuse tumors of the meninges 583

Brandes, W. C. See Simonds and Brandes 209, 273

Broun, G. O. See Peabody and Broun 169

Bunting, C. H., Bailey, G. H., and Davidson, P. B. The action of germanium dioxide on the rabbit 541

C

Cameron, Gordon, and Fitzpatrick, A. S. The hydatid cyst 227
Chobot, Robert. See Von Glahn and Chobot 373
Clawson, B. J. Rheumatic endocarditis as related to subacute bacterial endocarditis 542
——. See Bell and Clawson. 540
——. See Bell, Clawson and Hartzell 247
Crowell, C. See Krumbhaar and Crowell 544

D

Davidoff, Leo M. See Bailey and Davidoff 185
Davidson, P. B. See Bunting, Bailey and Davidson 541
Day, H. A. See Foot and Day 431

E

Elliott, Arthur R. See Hirsch and Elliott 627

F

Feldman, Wm. H. An adenocarcinoma resembling the thyroid gland . . 281
Fitzpatrick, A. S. See Cameron and Fitzpatrick 227
Foot, Nathan C. A Correction. 681
——. Studies on endothelial reactions. IX. The formation of reticulum in the lesions of experimental tuberculosis in rabbits 341
—— and Day, H. A. The occurrence of reticulum in tumors. 431

G

Goodpasture, Ernest W. The axis-cylinders of peripheral nerves as portals of entry to the central nervous system for the virus of herpes simplex in experimentally infected rabbits 11
——. Certain factors determining the incidence and severity of herpetic encephalitis in rabbits 47
——. Intranuclear inclusions in experimental herpetic lesions of rabbits . 1
——. The pathways of infection of the central nervous system in herpetic encephalitis of rabbits contracted by contact; with a comparative comment on medullary lesions in a case of human poliomyelitis . . 29
——. A study of rabies, with reference to a neural transmission of the virus in rabbits, and the structure and significance of Negri bodies . 547

H

Haden, Russell L. Intestinal obstruction and pernicious anemia. Report of a case 542
Hall, Ernest M., and Ophüls, W. Progressive alcoholic cirrhosis. . . . 477
——. See Tainter and Hall 503
Hartzell, T. B. See Bell, Clawson and Hartzell 247
Helwig, Ferdinand C. Arachnid infection in monkeys 389

Hirano, Norimasa. Researches on vaccine virus *in vitro* with special references to its affinity for nerve tissue 635
Hirsch, Edwin F., and Elliott, Arthur R. Ependymomas of the lateral and fourth ventricles of the brain 627
Howe, Percy. See Wolbach and Howe 535, 536

I

Ikeda, Kano. Studies of the blood in an epidemic of smallpox 542

J

Jacobson, Victor C. The fibroma of the acoustic nerve 259
——. Experimental endometriosis 537
Jaffe, H. L. See Marmorston-Gottesman and Jaffe 536

K

Karsner, Howard T.; Saphir, Otto, and Todd, T. Wingate. The state of the cardiac muscle in hypertrophy and atrophy 351
Killian, J. A. See MacNeal and Killian 537
Kline, B. S. The origin of adenomatous goitre 235
Kolodny, Anatole. Tissue changes after experimental deep roentgen irradiation 285
——. Congenital sarcoma of the leg (bone sarcoma?). Report of a case with eight years' cure 533
Krumbhaar, E. B., and Crowell, C. Spontaneous rupture of heart based on thirteen unpublished cases and six hundred and twenty from the literature 544

L

Laughlen, G. F. Studies on pneumonia following naso-pharyngeal injections of oil 407
Levin, Isaac, and Barnet, Joseph. Unusual modes of dissemination of ovarian carcinoma 534
Lewis, Margaret Reed. The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood 91

M

MacNeal, W. J., and Killian, J. A. Chemical studies on polychrome methylene blue 537
——. See Baylis, Sheplar and MacNeal 529
Mallory, F. B. The relation of chronic poisoning with copper to hemochromatosis 117
—— and Parker, Frederic, Jr. Experimental intracapillary glomerulonephritis 541
——. See Rowen and Mallory 677
Marmorston-Gottesman, J., and Jaffe, H. L. Compensatory hypertrophy of the thymus gland 536

McJunkin, Frank A. The origin of the mononuclear phagocytes of peritoneal exudates 305
McKee, S. Hanford. Epithelial cells in conjunctival infections 536
Meeker, Louise H. Riedel's struma associated with remnants of the postbranchial body 57
——. Plexiform neuroma of the pharyngeal mucous membrane 533
Meleney, Henry Edmund. The histopathology of kala-azar in the hamster, monkey and man 147
Menten, Maud L. The pathological effects of soluble toxic substances obtained from B. paratyphosus B. 539
Mentzer, Stanley H. Cholesterosis of the gall bladder 383
——. The pathogenesis of biliary calculi 538
Miller, A. James. Congenital heart disease with partial situs inversus, absence of inferior vena cava, and other anomalies 467
Milliken, Gibbs, and Poindexter, C. A. Mucocele of the appendix with globoid body formation 397

N

Nicholes, J. K. See Schultz, Nicholes and Schaefer 101
Nye, Robert N. See Parker and Nye. 325, 337

O

Ophüls, W. See Hall and Ophüls 477

P

Pappenheimer, Alwin M. See Von Glahn and Pappenheimer 445
Parker, Frederic, Jr., and Nye, Robert N. Studies on filterable viruses, I 325
—— and ——. Studies on filterable viruses, II 337
——. See Mallory and Parker 541
Peabody, Francis W., and Broun, G. O. Phagocytosis of erythrocytes in the bone marrow, with special reference to pernicious anemia . . . 169
Penfield, Wilder. Microglia and the process of phagocytosis in gliomas. 77
Plaut, Alfred. Bilharzia in the appendix 532
Poindexter, C. A. See Milliken and Poindexter 397

R

Ritter, Saul Alfred. Neuroblastoma of the intestine 519
Robinson, W. L. See Boyd and Robinson 135
Root, Howard F. See Warren and Root 415
Rosenau, M. J., and Bailey, G. H. Diphtheria immunity. The effect of repeated injections of avirulent diphtheria bacilli, B. Hofmanni and B. Xerosis in guinea-pigs 529
Rosenow, E. C. Localization of streptococci from cases of epidemic nausea and vomiting and of epidemic neuro-myelo-encephalitis . . 539
Rowen, H. S., and Mallory, F. B. A multinucleated liver cell carcinoma. 677

S

Saleeby, E. R. Heterotopia of the bone marrow without apparent cause. 69
Saphir, Otto. The effect of certain metabolic changes upon the aorta of rabbits and guinea-pigs 403
——. See Karsner, Saphir and Todd 351
Schaefer, J. H. See Schultz, Nicholes and Schaefer 101
Schultz, Edwin W. Hematocele of the wall of the atrium following cardiac puncture 225
——, Nicholes, J. K., and Schaefer, J. H. Studies on blood fibrin . . . 101
Seecof, David P. Studies on mitochondria 295
Sheplar, A. E. See Baylis, Sheplar and MacNeal 529
Simonds, J. P., and Brandes, W. C. The pathology of the hypophysis. I. The presence of abnormal cells in the posterior lobe 209
—— and——. The pathology of the hypophysis. II. Lymphocytic infiltration 273
Stewart, Fred W. Observations on focal tuberculin hypersensitiveness in an infected organ previous to a general sensitization 495
Stillman, Ralph G. A contribution to the knowledge of splenic anemia. 541

T

Tainter, M. L., and Hall, E. M. Gross and histologic changes in the edema of paraphenylenediamine 503
Todd, T. Wingate. See Karsner, Saphir and Todd 351

V

Von Glahn, William C., and Chobot, Robert. The histological alterations of the pancreas in chronic passive congestion 373
——. and Pappenheimer, Alwin M. Intranuclear inclusions in visceral disease 445

W

Warren, Shields. The etiology of acute appendicitis. 241
—— and Root, Howard F. The pathology of diabetes, with special reference to pancreatic regeneration 415
Wolbach, S. B., and Howe, Percy. The effect of the scorbutic state upon the production and maintenance of intercellular substances 535
—— and ——. The epithelial tissues in experimental xerophthalmia . 536
Wollstein, Martha. Rénal neoplasms in young children 534

Z

Zeckwer, Isolde T. Rapid alterations in the blood sugar level of rabbits in anaphylaxis and following injections of bacterial proteins 531
Zingher, Abraham. Further results with the Dick test and active immunization with scarlatinal toxin 530